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FRESH FISH —
QUALITY AND QUALITY CHANGES

FAO Fisheries Series *No. 29*

Fresh fish — quality and quality changes

a training manual
prepared for the
FAO/DANIDA Training Programme on
Fish Technology and Quality Control

by

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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
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Preface

Fresh fish — quality and quality changes is designed primarily for courses in fish technology. It was published first in Danish in 1983 under the title *Fersk fisk — kvalitet og holdbarhed* and constitutes part of the teaching material used by the Technical Laboratory, Ministry of Fisheries at the Technical University, Denmark.

The present issue in English is in part a translation of the Danish publication but it has been expanded with data relevant to tropical fisheries and tropical fish species. In this form it is intended as a basic text on the subject of fish technology for those who are undergoing training at the workshops run by FAO and financed by the Danish International Development Agency (DANIDA). DANIDA also agreed to fund the preparation of this publication.

Some of the material in this manual has already been used at a number of FAO/DANIDA workshops, and it is intended that this and future updated issues will be widely used for years to come. French and Spanish versions are now in preparation.

The Food and Agriculture Organization acknowledges the valuable assistance so generously given to the author by many collaborators in the preparation of this document.

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1. Aquatic resources and their utilization

More than two-thirds of the world's surface is covered by water. The oceans alone account for 71 percent. Tiny microscopic plants, the phytoplankton, are the primary producers of organic material using the energy supplied by the sun as shown in Figure 1.1.

This enormous primary production is the first link in the food chain and forms the basis for all life in the sea. How much harvestable fish results from this primary production has been the subject of much

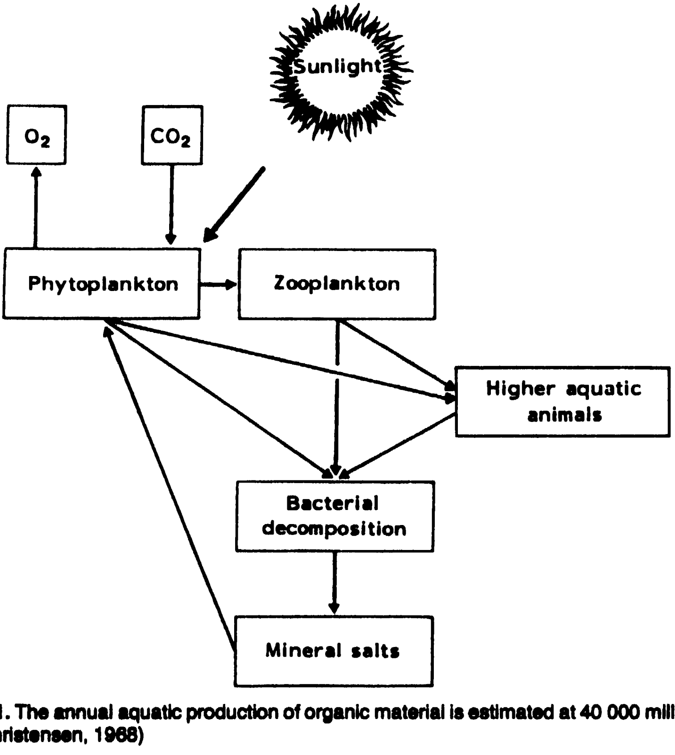


Figure 1.1. The annual aquatic production of organic material is estimated at 40 000 million tonnes (Møller Christensen, 1968)

speculation. However, there are great difficulties in estimating the ecological efficiency, i.e. the ratio of total production at each successive trophic level. Gulland (1971) reports a range from 10 to 25 percent but suggests 25 percent as the absolute upper limit of ecological efficiency; for example, not all of the production at one trophic level is consumed by the next. Ecological efficiency also varies between levels,

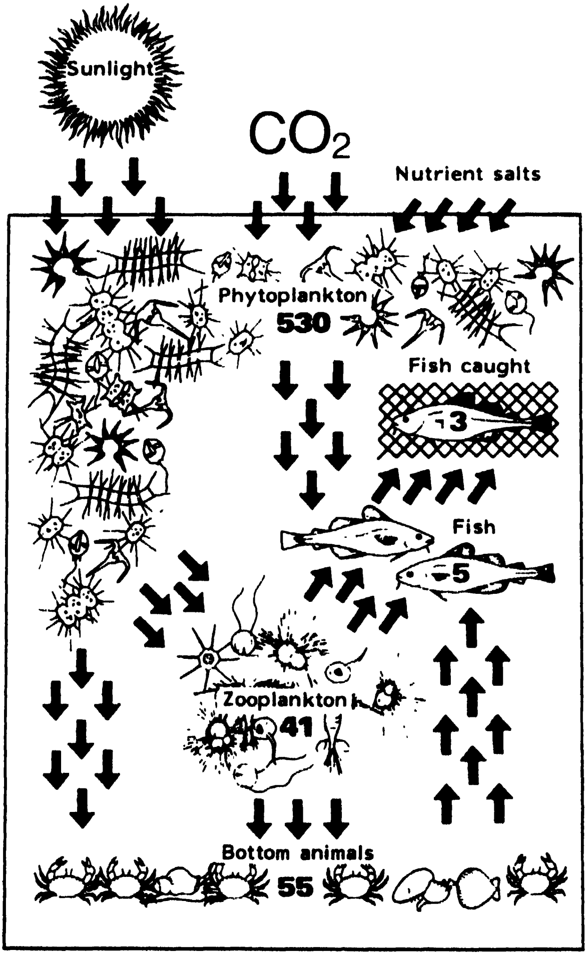


Figure 1.2. Annual production (in million tonnes) in the North Sea, one of the richest fishing waters in the world

being higher at the lower levels of the food chain with smaller organisms using proportionally more of their food intake for growth rather than for maintenance. Diseases, mortality, pollution, etc. may also influence ecological efficiency. The conditions in the North Sea, with very rich fishing waters, are shown in Figure 1.2 as an example.

Since production is greater in the early stages of the food chain, the potential catch is also greater if harvesting is carried out at these stages. If progress in catch handling, technology and marketing makes harvesting at the early trophic levels feasible, then the potential catch may increase accordingly (see Table).

The world catch stagnated in the 1970s at around 70 million tonnes but has now increased to 75 million tonnes (1984), including 9 million tonnes from freshwater sources. About 70 percent of this amount is used for food.

FAO sources estimate that the conventional catch of marine species could be increased by 20-30 million tonnes, much of which (50 percent) depends on improved management. Reduction of post-harvest losses and recovery of by-catches could add another 10-20 million

Summary of current and potential global aquatic resources

	World harvest 1981 (million tonnes)					Potential harvest increase	
	Used for food	Post-harvest losses	Industrial use	Total harvest landed	Discards at sea	Short term	Long term
Conventional							
Freshwater	7.9	0.2		8.1		4.5	[1]30
Marine: demersal	13.5	1.5	7.0	22.0	4-5	5	
small pelagic	15.6	3.2	14.6	33.4		5	15
others	3.1	0.1	+	3.2		2	
shellfish	5.9	0.2		6.1		0.7	[1]?
Unconventional							
Cephalopods	1.3	+	0.1	1.4		4	150+
Mesopelagic (Lantern fish)	?	+	+	+		+	100+
Euphausids (Krill)	+		0.4	0.4		+	50+
Seaweeds	1.8		1.2	3.0		8	[1]?
Total	**49.1**	**5.2**	**23.3**	**77.6**	**4-5**	**29.2**	**345+**

[1] Magnitude depends on culture.
Source: Whittle, 1984.

tonnes. The freshwater aquaculture potential is not known but could possibly yield up to 30 million tonnes in the future. However, to meet the demand, it is essential to reduce the proportion of catch used for industrial purposes and develop unconventional resources such as those shown in the Table. The use of food fish is shown in Figure 1.3.

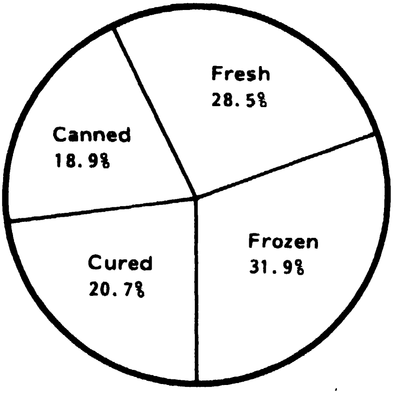


Figure 1.3. Food fish usage, 1982 (James, 1984)

It has been estimated by James (1984) that about 8 percent of the food fish never reaches the market, which means that, in 1982, 4.25 million tonnes were wasted. These post-harvest losses are most significant in the fresh and cured fish sectors. The important thing is that the technology for limiting or preventing these losses is available but needs to be put into effect, as pointed out by James (1984).

2. Classification, anatomy and physiology of fish

Classification

Fish are the most numerous of the vertebrates with 20 000 species known and probably many more unknown (Lagler *et al.*, 1977).

Fish are usually divided into three classes: Cephalaspidomorphi, jawless fish like lampreys and slime-eels; Chondrichthyes, cartilaginous fish like sharks and rays; and Osteichthyes, lungfish and all other bony fish, which include most of the commercially important species. These classes represent numerous genera, which are then further subdivided into different species.

The use of common or local names often creates confusion since the same species may have different names in different regions or, conversely, the same name is ascribed to several different species, sometimes with different technological properties. As a point of reference the scientific name should, therefore, be given in any kind of publication or report the first time a particular species is referred to by its common name. It is normally written in italics (or underlined). For further information see the ICES "List of Names of Fish and Shellfish", *Bulletin statistique*, vol. 49, September 1966; the Torry Advisory Note No. 55, *Fish Names in the Common Market*; and the *Multilingual Dictionary of Fish and Fish Products* prepared by OECD and published by Fishing News Books Ltd, United Kingdom.

The classification of fish into cartilaginous and bony (the jawless fish are of minor importance) is important from a practical viewpoint, since these groups of fish spoil differently (Chapter 4) and vary with regard to chemical composition (Chapter 3). Most non-taxonomists refer to these groups as teleosts (bony fish) and elasmobranchs (cartilaginous fish).

Furthermore, fish can be divided into fatty and lean species, but this type of classification is based purely on some technological characteristics.

Anatomy and physiology

The skeleton

Being vertebrates, fish have a vertebral column — the backbone — and a cranium covering the brain. The backbone runs from the head to the tail fin and is composed of segments (vertebrae). These vertebrae are extended dorsally to form neural spines and in the trunk region they have lateral processes that bear ribs (Fig. 2.1). The ribs are cartilaginous or bony structures in the connective tissue (myocommata) between the muscle segments (myotomes) (see also Fig. 2.2). Usually, there is also a corresponding number of false ribs or "pin bones" extending more or less horizontally into the muscle tissue. These bones cause a great deal of trouble when fish are being filleted or otherwise prepared for food.

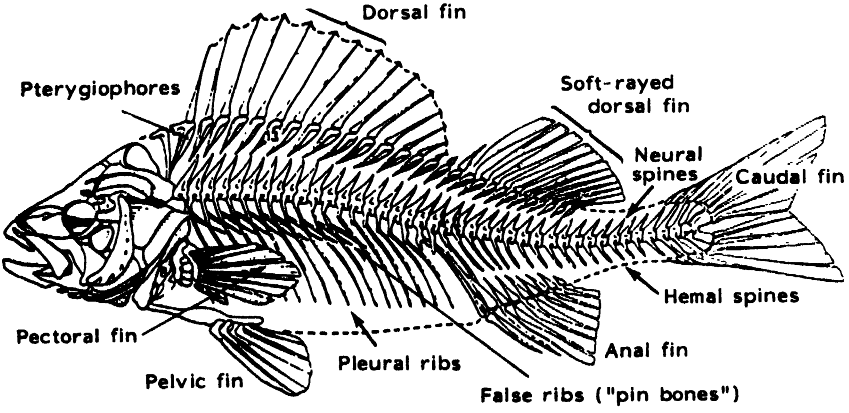


Figure 2.1. Skeleton of bony fish (Eriksson and Johnson, 1979)

Muscle anatomy and function

The anatomy of fish muscle is very simple. Basically, there are two bundles of muscles on each side of the vertebral column and each of these bundles is further separated into an upper mass above the hori-

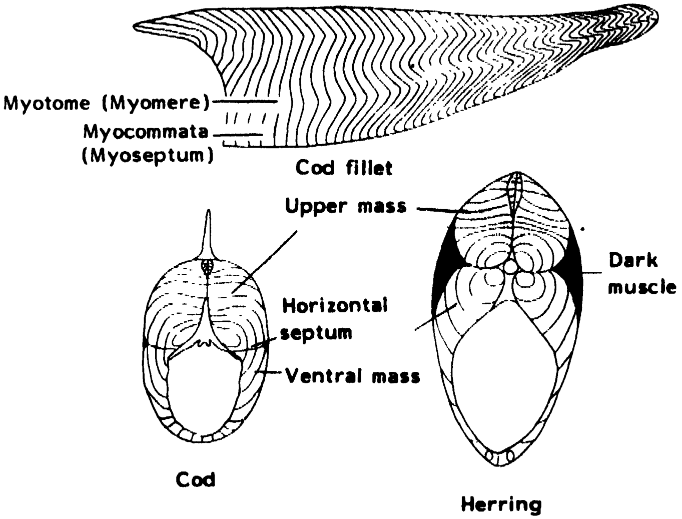
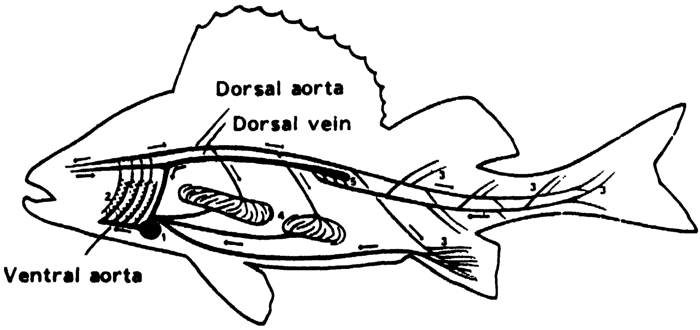


Figure 2.2. Skeletal musculature of fish (Knorr, 1974)

zontal axial septum and a ventral mass below this septum. The muscle cells run in a longitudinal direction separated perpendicularly by sheets of connective tissue (myocommata). The muscle segments lying in between the sheets of connective tissue are called myotomes. The longest muscle cells are found in the twelfth myotome counting from the head (Fig. 2.2) and the average length of these is around 10 mm in a cod that is 60 cm long (Love, 1970). The length of the cells, as well as the thickness of the myocommata, will increase with age.

With this anatomy the fish muscle tissue contains comparatively far less connective tissue than mammalian muscle (Chapter 3).

As in mammals, the muscle tissue of fish is composed of striated muscle. The functional unit, i.e. the muscle cell, consists of sarcoplasma containing nuclei, glycogen grains, mitochondria, etc. and a number (up to 1 000) of myofibrils. The cell is surrounded by a sheath of connective tissue called the sarcolemma. The myofibrils contain the contractile proteins, actin and myosin. These proteins or filaments are arranged in a characteristic alternating way making the muscle look striated upon microscopic examination (Fig. 2.3).



1. The heart pumps blood toward the gills.
2. The blood is aerated in the gills.
3. Arterial blood is dispersed into the capillaries where the transfer of oxygen and nutrients to the surrounding tissue takes place.
4. The nutrients from ingested food are absorbed from the intestines, then transported to the liver and later dispersed in the blood throughout the body.
5. In the kidneys, the blood is "purified" and waste products are excreted via the urine.

Figure 2.4. Blood circulation in fish (Eriksson and Johnson, 1979)

30 mg Hg in the ventral aorta to 0 when entering the heart (Johansen, 1962 cf. Randall, 1970). After the blood has passed through the gills, the blood pressure derived from the pumping activity of the heart is already greatly decreased. Muscle contractions are important in pumping the blood back to the heart and counterflow is prevented by a system of paired valves found inside the veins.

Clearly, the single circulation of fish is fundamentally different from the system in mammals (Fig. 2.5), where the blood passes through the heart twice and is propelled out into the body under high pressure due to the contractions of the heart.

In fish, the heart does not play an important role in the transportation of blood from the capillaries back to the heart. This has been confirmed in an experiment where the impact of different bleeding procedures on the colour of cod fillets was examined. No difference could be

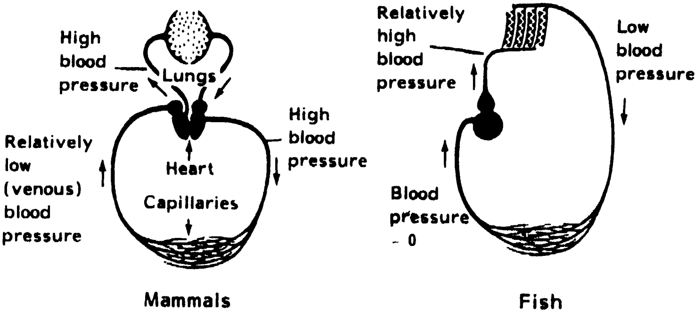


Figure 2.5. Blood circulation in fish and mammals (Eriksson and Johnson, 1979)

found regardless of whether the fish had been bled by means of cutting the throat in front of or behind the heart before gutting, or it had not been cut at all before slaughter. Better bleeding was obtained when a deep throat cut (including the dorsal aorta) was employed followed by immediate gutting. It is of major importance to cut the fish while it is either still alive or at least in a pre-rigor state since it is the muscle contractions (which includes rigor contractions) that force the blood out of the tissues (Huss and Asenjo, 1977a). The total volume of the blood in fish ranges from 1.5 to 3.0 percent of the body weight. Most of it is located in the internal organs while the muscular tissues, constituting two-thirds of the body weight, contain only 20 percent of the blood volume. This distribution is not changed during exercise since the light muscle in particular is not very vascularized.

Other organs

Among the other organs, only the roe and liver play a major role as foodstuffs. Their size depends on the fish species and varies with life cycle, feed intake and season. In cod, the weight of the roe varies from a few percent up to 27 percent of the body weight and the weight of the liver ranges from 1 to 4.5 percent. Likewise, the composition can change and the oil content of the liver vary from 15 to 75 percent, with the highest values being found during autumn (Jangaard *et al.*, 1967).

Growth and reproduction

During growth, it is the size of each muscle cell that increases rather than the number of muscle cells. Also, the proportion of connective tissue increases with age.

Most fish become sexually mature when they reach a certain size which is characteristic of the species and is not necessarily directly correlated with age. In general, this critical size is reached earlier in males than in females. As the growth rate decreases after the fish has reached maturity, it is therefore often an economic advantage to rear female fish in aquaculture.

Every year, mature fish use energy to build up the gonads (the roe and milt). This gonadal development causes a depletion of the protein and lipid reserves of the fish since it takes place during a period of low or no food intake (Fig. 2.6). In North Sea cod, it was found that prior

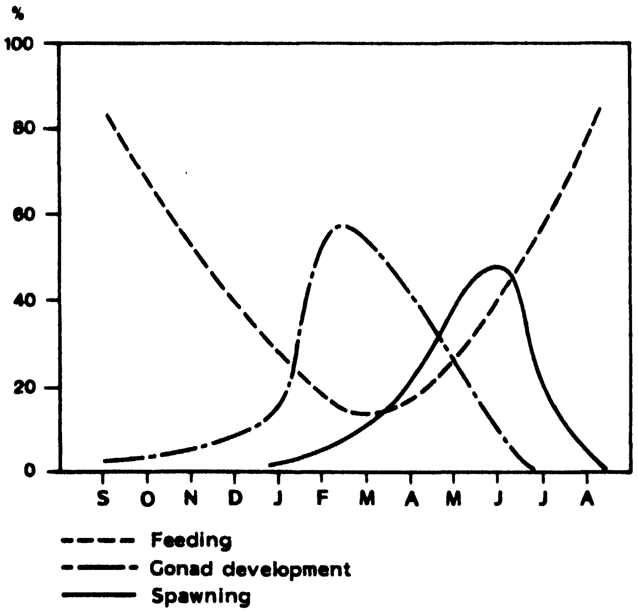


Figure 2.6. Relation between feeding cycle (percent sample with food in stomach) and reproductive cycle (gonad development), percent fish with ripening gonads (spawning, percent ripe fish) of haddock (*Melanogrammus aeglefinus*). It should be noted that the development of the gonads takes place while the fish is starving (Hoar, 1957).

to spawning the water content of the muscle increases (Fig. 2.7) and the protein content decreases. In extreme cases, the water content of very large cod can attain 87 percent of the body weight prior to spawning (Love, 1970).

The length of the spawning season varies greatly between species. Most species have a marked seasonal periodicity (Fig. 2.6), while some have ripe ovaries for nearly the whole year.

The depletion of the reserves of the fish during gonadal development can be extremely severe especially if reproduction is combined with migration to the breeding grounds. Some species, for example Pacific salmon (*Oncorhynchus* sp.), eel (*Anguilla anguilla*) and others, manage to migrate only once, after which they degenerate and die. This is partly because these species do not eat during migration so that, in the case of a salmon, it can lose up to 92 percent of its lipid, 72 percent of its protein and 63 percent of its ash content during migration and reproduction (Love, 1970).

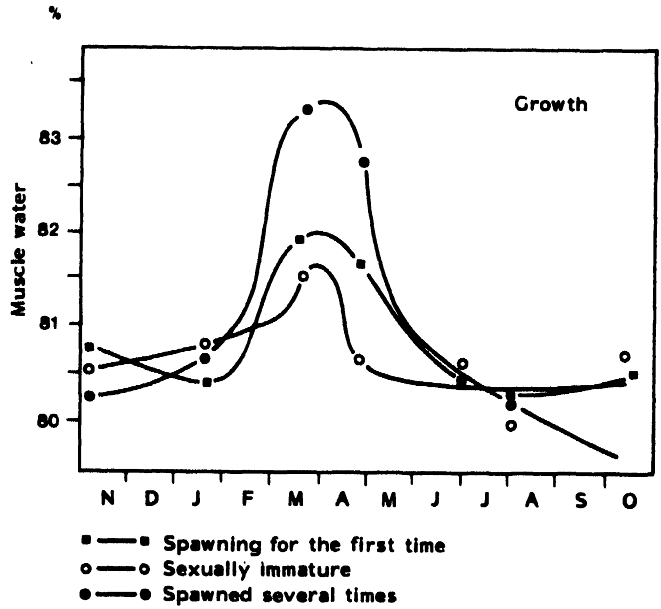


Figure 2.7. Water content of cod muscle (*Gadus morhua*) (Love, 1970)

On the other hand, other fish species are capable of reconstituting themselves completely after spawning for several years. The North Sea cod lives for about eight years before spawning causes its death, and other species can live even longer (Cushing, 1975). In former times, 25-year-old herring (*Clupea harengus*) were not unusual in the Norwegian Sea and plaice (*Pleuronectes platessa*) up to 35 years old have been found. One of the oldest fish reported was a sturgeon (*Acipenser sturio*) from Lake Winnebago in Wisconsin. According to the number of rings in the earstone, it was over 100 years old.

3. Chemical composition

Principal constituents

The chemical composition of fish varies greatly from species to species and also from individual to individual depending on age, sex, environment and season.

The principal constituents of fish and mammals are the same and examples of the variation between them are shown in Table 3.1.

The variations in the chemical composition of fish are closely related to the feed intake. During periods of heavy feeding, at first the protein content of the muscle tissue will increase very slightly and then the lipid content will show a marked and rapid increase. Fish will have starvation periods for natural or physiological reasons (such as spawning or migration) or because of external factors such as shortage of food. In the case of the latter, plankton-eating species such as herring will naturally experience a seasonal variation since plankton production depends on the season.

The lipid fraction is the component showing the greatest variation. Often the variation within a certain species will display a characteristic seasonal curve with a minimum around the time of the spawning. Figure 3.1 shows the characteristic variations in North Sea herring (3.1a) and mackerel (3.1b).

TABLE 3.1. **Principal constituents (percent) of fish and beef muscle**

Constituent	Fish (fillet)			Beef (isolated muscle)
	Min	Normal variation	Max	
Protein	6	16 -21	28	20
Lipid	0.1	0.2-25	67	3
Carbohydrate		< 0.5		1
Ash	0.4	1.2- 1.5	1.5	1
Water	28	66 -81	96	75

Sources: Stansby, 1962; Love, 1970; Bendall, 1962.

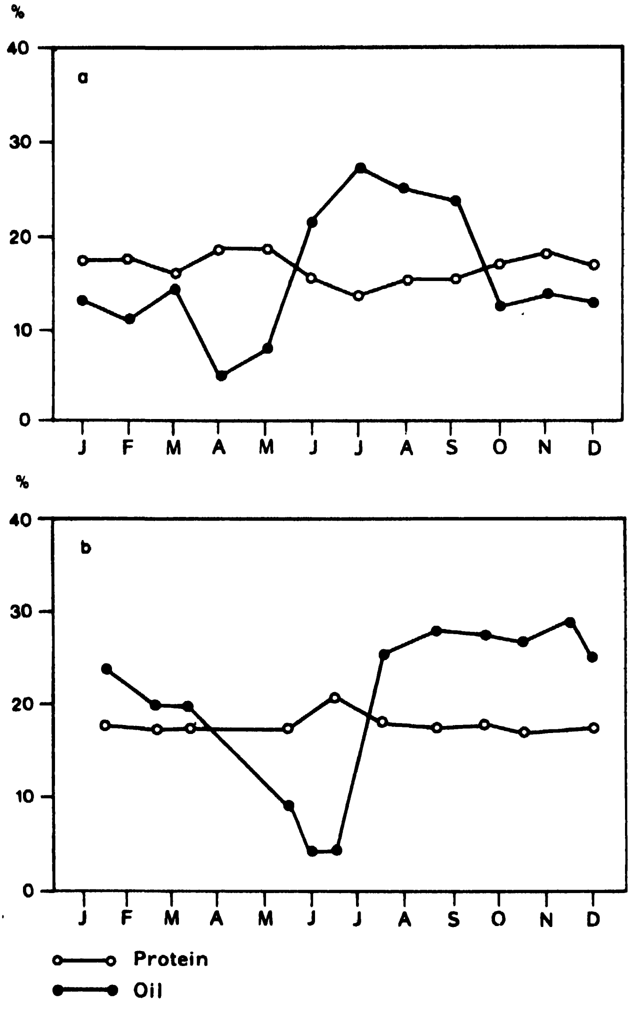


Figure 3.1. Seasonal variation in the chemical composition of (a) herring (*Clupea harengus*), and (b) mackerel fillets (*Scomber scombrus*). Each point indicates the mean value of eight fillets

However, some tropical fish also show a marked seasonal variation in chemical composition. West African shad (*Ethmalosa dorsalis*) show a range in fat content of 2-7 percent (wet weight) over the year with a maximum in July (Watts, 1957). Corvina (*Micropogon furnieri*) and pescada-foguete (*Marodon ancylodon*) captured off the Brazilian coast had a fat content of 0.2-8.7 percent and 0.1-5.4 percent, respectively (Ito and Watanabe, 1968). It has also been observed that the oil content of these species varies with size, larger fish containing about 1 percent more oil than smaller ones. Watanabe (1971) examined freshwater fish from Zambia and found a variation from 0.1 to 5.0 percent in oil content of four species including both pelagic and demersal ones.

The variation in the percentage of fat is reflected in the percentage of water, since fat and water normally constitute around 80 percent of the fillet.

The technological characteristics of fish are mainly influenced by the lipid content and for this reason it has been convenient to classify fish as fatty (e.g. herring, mackerel, tuna and sprat), semi-fatty (e.g. barracuda, mullet and shark) or lean (e.g. cod, hake and plaice) species. The lean species store most of their fat in the liver. Some variations in the percentage composition of different fish species are shown in Table 3.2.

TABLE 3.2. **Chemical composition of the fillets of various fish species**

Species	Scientific name	Water %	Lipid %	Protein %	Energy value (kJ/100 g)
Blue whiting	*Micromesistius poutassou*	79-80	1.9- 3.0	13.8-15.9	314-388
Cod	*Gadus morhua*	78-83	0.1- 0.9	15.0-19.0	295-332
Eel	*Anguilla anguilla*	60-71	8.0-31.0	14.4	
Herring	*Clupea harengus*	60-80	0.4-22.0	16.0-19.0	
Plaice	*Pleuronectes platessa*	81	1.1- 3.6	15.7-17.8	332-452
Salmon	*Salmo salar*	67-77	0.3-14.0	21.5	
Trout	*Salmo trutta*	70-79	1.2-10.8	18.8-19.1	
Tuna	*Thunnus* sp.	71	4.1	25.2	581
Norway lobster	*Nephrops norvegicus*	77	0.6- 2.0	19.5	369

Source: Murray and Burt, 1969.

Lipids

When the lipid content in the fish exceeds 1 percent it functions as an energy reserve and can be classified as fat depots. The fat depots are mostly located in the subcutaneous tissue, in the belly flap, in the collagenous tissue between the muscle fibres both in light and dark muscle, and in the head section. However, it should be noted that also in this aspect marked differences between fish species are found as demonstrated in Figure 3.2.

In most fish species the fat depots consist of triglycerides as in most other vertebrates. However, in some fish species, these triglycerides are found together with or even replaced by other types of lipids. In cartilaginous fish such as sharks, a significant quantity of the fat may consist either of diacyl alkyl glyceryl ethers or of the hydrocarbon squalene.

Fish lipids differ from mammalian lipids. The main difference is that they consist of long-chain fatty acids (14-22 carbon atoms) which are highly unsaturated. Mammalian fat will rarely contain more than two

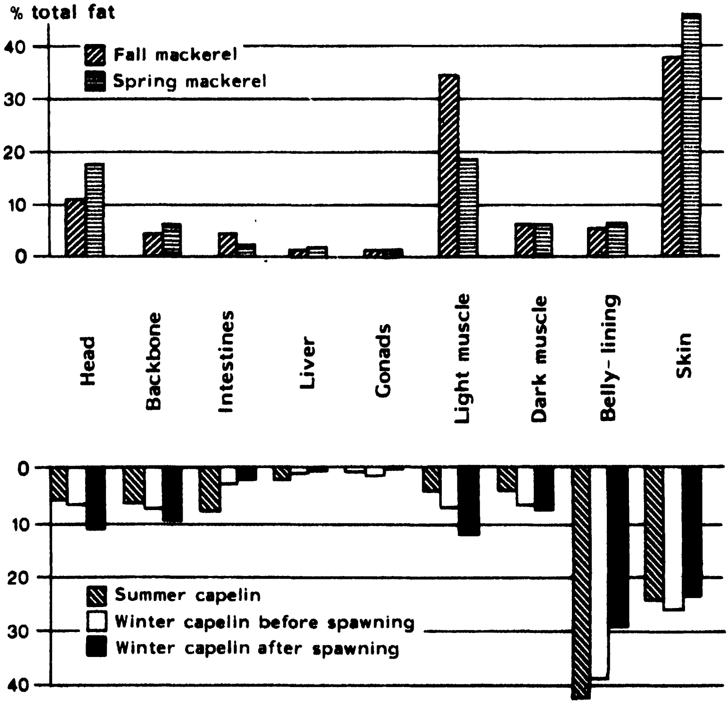


Figure 3.2. Distribution of the total fat in various parts of the body of mackerel (top) and capelin (bottom) of Norwegian origin (Lohne, 1976)

double bonds per fatty acid molecule while the depot fats of fish contain many fatty acids with five or six double bonds (Stansby and Hall, 1967).

The total number of polyunsaturated fatty acids with four, five or six double bonds is slightly lower in lipids from freshwater fish (approximately 70 percent) than in lipids from marine fish (approximately 88 percent), (Stansby and Hall, 1967). However, the composition of the lipids is not completely fixed but can vary somewhat with the feed intake.

In human nutrition some fatty acids like linoleic and linolenic acid are regarded as essential since they cannot be synthesized by the organism. In fish, these fatty acids constitute only around 2 percent of the total lipids, which is a small percentage compared with many vegetable oils. However, fish oils contain other polyunsaturated fatty acids which can cure skin diseases in the same way as linoleic and arachidonic acid. As members of the linolenic acid family (first double bond in the third position, ω-3, counted from the terminal methyl group), they will also have a growth-promoting effect on growing children. The fatty acid, eicosapentaenoic acid (C20:5ω3), has recently attracted considerable attention because some Danish scientists found this acid in the blood and diet of a group of Greenland Eskimos virtually free from atherosclerosis. Investigations in the United Kingdom have documented that eicosapentaenoic acid is an extremely potent antithrombotic factor.

A small portion of the lipids (less than 1 percent of the fish muscle) serves as essential structural parts of the cell. Typical "non-depot lipids" are the phospholipids which are phosphorus- and nitrogen-containing fats. Usually, these lipids do not function as an energy reserve; however, in white-fleshed fish such as cod that have no fat depots in the muscle tissue, some of the phospholipids will be used during longer starvation periods (Love, 1980).

Japanese researchers have shown that the phospholipids are distributed unevenly in the tissues, with dark muscle in particular being rich in these compounds. A large percentage of the fatty acids in the phospholipids are also long-chain polyunsaturated acids with five or six double bonds. In most fish species the phospholipids constitute approximately 0.5-1 percent of the muscle tissue.

The main sterol in fish muscle is cholesterol, found in quantities generally well under 100 mg/100 g, which are not much higher than those found in mammalian muscle tissue. Excellent reviews on fish lipids are given by Ackman (1980) and Malins and Wekell (1970).

Proteins

The proteins in fish muscle tissue can be divided into the following three groups:

1. Structural proteins (actin, myosin, tropomyosin and actomysin), which constitute 70-80 percent of the total protein content (compared with 40 percent in mammals). These proteins are soluble in neutral salt solutions of fairly high ionic strength ($\geqslant$0.5 M).
2. Sarcoplasmic proteins (myoalbumin, globulin and enzymes) which are soluble in neutral salt solutions of low ionic strength (<0.15 M). This fraction constitutes 25-30 percent of the protein.
3. Connective tissue proteins (collagen), which constitute approximately 3 percent of the protein in teleosteii and about 10 percent in elasmobranchii (compared with 17 percent in mammals).

The isoelectric point of fish proteins is around pH 4.5-5.5. At this pH the proteins are electrically neutral and less hydrophilic than in the ionized state, which means that their water-binding capacity and solubility are at a minimum. If the pH is higher or lower than the isoelectric point, the solubility will increase (Fig. 3.3).

Fish proteins contain all the essential amino-acids and, like milk, eggs and mammalian meat proteins, have a very high biological value (Table 3.3).

Cereal grains are usually low in lysine and/or the sulphur-containing amino-acids (methionine and cysteine), whereas fish protein is an excellent source of these amino-acids. In diets based mainly on cereals, a supplement of fish can, therefore, raise the biological value significantly.

N-containing extractives

The N-containing extractives can be defined as the water-soluble, low molecular weight, nitrogen-containing compounds of non-protein nature. This NPN-fraction (non-protein nitrogen) constitutes from 9 to 18 percent of the total nitrogen in teleosts.

The major components in this fraction are: volatile bases such as ammonia and trimethylamine oxide (TMAO), creatine, free amino-acids, nucleotides and purine bases, and, in the case of cartilaginous fish, urea.

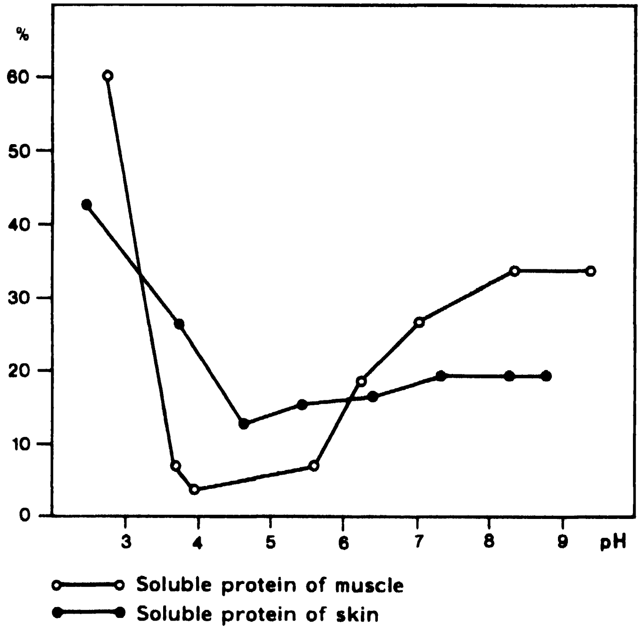


Figure 3.3. Soluble protein of muscle and skin at different pH values after incubation for 20 hou
8°C (Gildberg and Raa, 1979)

TABLE 3.3. **Essential amino-acids (percentage) in various proteins**

Amino-acid	Fish	Milk	Beef	Eggs
Lysine	8.8	8.1	9.3	6.8
Tryptophan	1.0	1.6	1.1	1.9
Histidine	2.0	2.6	3.8	2.2
Phenylalanine	3.9	5.3	4.5	5.4
Leucine	8.4	10.2	8.2	8.4
Isoleucine	6.0	7.2	5.2	7.1
Threonine	4.6	4.4	4.2	5.5
Methionine-cysteine	4.0	4.3	2.9	3.3
Valine	6.0	7.6	5.0	8.1

Sources: Braekkan, 1976; Moustgard, 1957.

Table 3.4 lists some of the components in the NPN-fraction of various fish, poultry meat and mammalian meat.

An example of the distribution of the different compounds in the NPN-fraction in freshwater and marine fish is shown in Figure 3.4. It should be noted that the composition varies not only from species to species but also within the species depending on size, season, muscle sample, etc.

TMAO constitutes a characteristic and important part of the NPN-fraction in marine species and deserves a further mention. This component is found in all marine fish species in quantities from 1 to 7 percent of the muscle tissue (dry weight) but is virtually absent from freshwater species and from terrestrial organisms. Although a considerable amount of work has been conducted on the origin and role of TMAO, there is still much to be clarified. Strøm (1979) has shown that TMAO is formed by biosynthesis in certain zooplankton species. These organisms possess an enzyme (TMA monooxygenase) which oxidizes TMA to TMAO. TMA is commonly found in marine plants as are many other methylated amines (monomethylamine and dimethylamine). Plankton-eating fish may obtain their TMAO from feeding on these

TABLE 3.4. **Major differences in muscle extractives**

Compound in mg/100 g wet weight[1]	**Fish**			**Crustaceans**	**Poultry**	**Mammalian muscle**
	Cod	**Herring**	**Shark sp.**	**Lobster**	**Leg muscle**	
1) Total extractives	1 200	1 200	3 000	5 500	1 200	3 500
2) Total free amino-acids:	75	300	100	3 000	440	350
Arginine	<10	<10	<10	750	<20	<10
Glycine	20	20	20	100-1 000	<20	<10
Glutamic acid	<10	<10	<10	270	55	36
Histidine	<1.0	86	<1.0	—	<10	<10
Proline	<1.0	<1.0	<1.0	750	<10	<10
3) Creatine	400	400	300	0	—	550
4) Betaine	0	0	150	100	—	—
5) Trimethylamine oxide	350	250	500-1 000	100	0	0
6) Anserine	150	0	0	0	280	150
7) Carnosine	0	0	0	0	180	200
8) Urea	0	0	2 000	—	—	35

[1] Note that the unit in this table refers to the total molecular weight of the compound.
Source: Shewan, 1974.

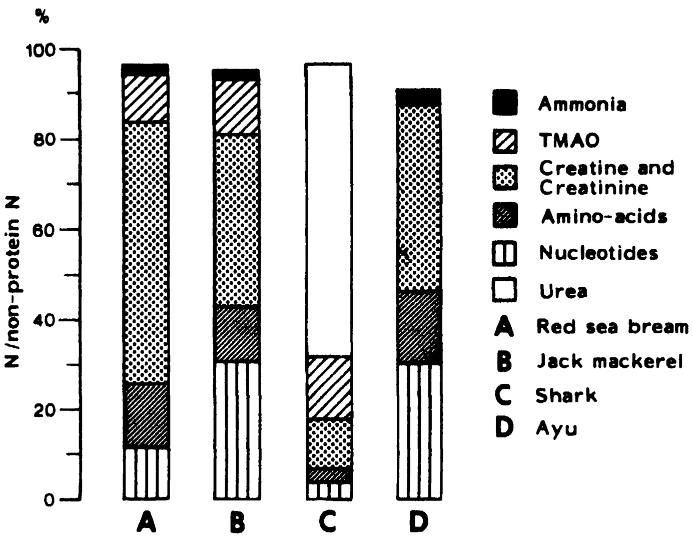


Figure 3.4. Distribution of non-protein nitrogen in fish muscles of two marine bony fish (A, B), an elasmobranch (C), and a freshwater fish (D) (Konosu and Yamaguchi, 1982; Suyama *et al.*, 1977)

zooplankton (exogenous origin). Belinski (1964) and Agustsson and Strøm (1981) have shown that certain fish species are able to synthesize TMAO from TMA, but this synthesis is regarded as being of minor importance.

The TMA-oxidase system is found in the microsomes of the cells and is NADPH-dependent:

$$(CH_3)_3N + NADPH + H^+ + O_2 \rightarrow (CH_3)_3NO + NADP^+ + H_2O$$

It is puzzling that this mono-oxygenase can be widely found in mammals (where it is thought to function as a detoxifier), while most fish have low or no detectable activity of this enzyme.

Japanese research (Kawabata, 1953) indicates that there is a TMAO-reducing system present in the dark muscle of certain pelagic fishes.

The amount of TMAO in the muscle tissue depends on the species, season, fishing ground, etc. In general, the highest amount is found in elasmobranchs and squid (75-250 mg N/100 g), cod have somewhat less (60-120 mg N/100 g) and flat and pelagic fish have the least. An extensive compilation of data is given by Hebard *et al.* (1982). According to Tokunaga (1970), pelagic fish (sardines, tuna, mackerel) have their highest concentration of TMAO in the dark muscle while demersal, white-fleshed fish have a much higher content in the white muscle.

In elasmobranchs, TMAO seems to play a role in osmoregulation, and it has been shown that a transfer of small rays to a mixture of fresh and sea water (1:1) will result in a 50 percent reduction of intracellular TMAO. The role of TMAO in teleosts is more uncertain.

Several hypotheses for the role of TMAO have been proposed:

1. TMAO is essentially a waste product, a detoxified form of TMA.
2. TMAO is an osmoregulator.
3. TMAO functions as an "anti-freeze".
4. TMAO has no significant function. It is accumulated in the muscle when the fish is fed a TMAO-containing diet.

According to Strøm (1984), it is now generally believed that TMAO has an osmoregulatory role.

Quantitatively, the main component of the NPN-fraction is creatine. In resting fish, most of the creatine is phosphorylated and supplies energy for muscular contraction.

The NPN-fraction also contains a fair amount of free amino-acids. These constitute 630 mg/100 g light muscle in mackerel (*Scomber scombrus*), 350-420 mg/100 g in herring (*Clupea harengus*) and 310-370 mg/100 g in capelin (*Mallotus villosus*). The relative importance of the different amino-acids varies with species. Taurine, alanine, glycine and imidazole-containing amino-acids seem to dominate in most fish. Of the imidazole-containing amino-acids, histidine has attracted much attention because it can be decarboxylated microbiologically to histamine. Active, dark-fleshed species such as tuna and mackerel have a high content of histidine.

The amount of nucleotides and nucleotide fragments in dead fish depends on the state of the fish and will be dealt with in Chapter 4.

Vitamins and minerals

The amount of vitamins and minerals is species-specific and can furthermore vary with season. In general, fish meat is a good source of the B vitamins and, in the case of fatty species, also of the A and D

TABLE 3.5. **Vitamins in fish**

Fish	**A** (IU/g)	**D** (IU/g)	**B_1 (thiamine)** (μg/g)	**B_2 (riboflavin)** (μg/g)	**Niacin** (μg/g)	**Pantothenic acid** (μg/g)	**B_6** (μg/g)
Cod fillet	0-50	0	0.7	0.8	20	1.7	1.7
Herring fillet	20-400	300-1 000	0.4	3.0	40	10	4.5
Cod-liver oil	200-10 000	20-300	—	[1]3.4	[1]15	[1]4.3	—

[1] Whole liver.
Source: Murray and Burt, 1969.

TABLE 3.6. **Some mineral constituents of fish muscle**

Element	**Average value** (mg/100 g)	**Range** (mg/100 g)
Sodium	72	30 -134
Potassium	278	19 -502
Calcium	79	19 -881
Magnesium	38	4.5-452
Phosphorus	190	68 -550

Source: Murray and Burt, 1969.

vitamins. Some freshwater species such as carp have a high thiaminase activity so the thiamine content in these species is usually low. As for minerals, fish meat is regarded as a valuable source of calcium and phosphorus in particular but also of iron and copper. Saltwater fish have a high content of iodine. In Tables 3.5 and 3.6 some of the vitamin and mineral contents are listed. Because of the natural variation of these constituents, it is impossible to give accurate figures.

The vitamin content is comparable to that of mammals except in the case of the A and D vitamins, which are found in large amounts in the meat of fatty species and in abundance in the liver of species such as cod and halibut. It should be noted that the sodium content of fish meat is relatively low, which makes it suitable for low-sodium diets.

4. Post-mortem changes in fish

Organoleptic changes

Organoleptic changes are those perceived with the senses, i.e. appearance, odour, texture and taste.

Changes in raw fresh fish

The earliest changes are, in particular, those that concern appearance and texture and rigor mortis (Fig. 4.1).

Immediately after death the fish muscles are totally relaxed. The fish is soft and pliable, and the texture is firm and elastic to the touch. After some time the muscle tissue will contract. When it becomes hard and stiff and the whole body becomes inflexible, the fish is said to be in rigor mortis.

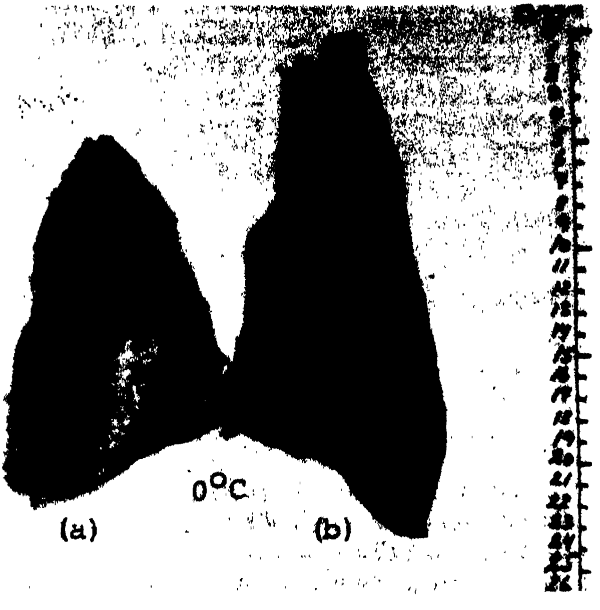


Figure 4.1. Fillets of cod (*Gadus morhua*) cut from the same fish before (a) and after (b) onset of rigor mortis. The shrinkage of fillet (a) was 24 percent

If the fish is filleted pre-rigor, the muscles can contract freely and the fillets will shorten and have a wrinkled surface. Dark muscle may shrink up to 52 percent and white muscle up to 15 percent of the original length (Buttkus, 1963). After rigor the muscle tissue returns to a relaxed state. With some experience, it is possible to distinguish between pre-rigor and post-rigor fish since pre-rigor fish are fully flexible (Trucco *et al.*, 1982) and do not show pressure marks after gentle squeezing.

The time involved in each stage of the development, duration and subsequent resolution of rigor mortis depends on many factors such as species, size, catching method, handling of the fish, temperature and the physical condition of the fish. In Table 4.1 some observations regarding the importance of different factors are listed.

It should be noted that exhausted fish (such as those which have been trawled) and fish kept at high temperatures will enter and pass through rigor quickly. Small and active, struggling fish do the same while large and flat fish in general take a longer time.

TABLE 4.1. **Onset and duration of rigor mortis in various fish species**

Species	**Condition**	**Temperature** (°C)	**Time from death to the onset of rigor** (hours)	**Time from death to the end of rigor** (hours)
Cod (*Gadus morhua*)	Trawled	0	2-8	20-65
Cod (*Gadus morhua*)	Trawled	10-12	1	20-30
Cod (*Gadus morhua*)	Trawled	30	0.5	1-2
Cod (*Gadus morhua*)	Rested	0	14-15	72-96
Grouper (*Epinephelus malabaricus*)	Rested	2	2	18
Tilapia (*Tilapia mossambica*) (small, 60 g)	Rested	0-2	2-9	26.5
Grenadier (*Macrourus whitsoni*)	Trawled	0	<1	35-55
Anchovy (*Engraulis anchoita*)	Trawled	0	20-30	18
Plaice (*Pleuronectes platessa*)	Trawled	0	7-11	54-55
Coalfish (*Pollachius virens*)	Trawled	0	18	110
Redfish (*Sebastes* spp.)	Trawled	0	22	120

Sources: Stroud, 1969; Partmann, 1965a; Nazir and Magar, 1963; Pawar and Magar, 1965; Trucco *et al.*, 1982.

Although it is generally accepted that the onset and duration of rigor mortis is more rapid at high temperatures, it has been observed in certain tropical fish that the biochemical changes, and thus rigor mortis, may actually be stimulated at 0°C compared with 22°C (Poulter *et al.*, 1982).

If rigor develops at high temperatures (in the case of cod this is above 17°C), the rigor tension may become very strong and can cause "gaping", i.e. weakening of the connective tissue and rupture of the fillet.

The technological significance of rigor mortis is of major importance when fish are frozen, especially in the case of fillets. If fish are filleted pre-rigor, as mentioned before, the fillets may shrink; if these fillets are frozen, they will often have a poor texture and enhanced drip loss. Fillets taken from fish in rigor are usually of good quality, but machine filleting is more difficult and results in a lower yield. Rough handling of fish in rigor will also cause gaping. In principle it is safer to fillet the fish post-rigor and freeze these fillets but often this is not possible as it requires a large chill storage capacity for the whole fish.

The post-mortem changes in appearance, texture and odour of whole wet fish are described in Table 4.2.

In general, off-odours can be detected first in the area around the abdominal cavity. In fish (such as herring and mackerel) which are not eviscerated after catch, this may happen long before the rest of the fish shows any sign of spoilage. In some cases high enzyme activity in the gut of fish caught during their feeding period can cause complete degradation of the belly and even make it burst. This phenomenon is called "belly burst" and may happen within a few hours of capture.

Changes in eating quality

The changes in the eating quality of chilled fish during storage can be assessed by daily organoleptic examination of the cooked flesh. Most often the assessment is carried out on poached fish as this cooking method allows most off-flavours to be detected. A characteristic pattern can be found (Phases 1-4).

Phase 1. The fish is very fresh with a species-specific taste and odour. Very often the flavour is seaweedy and delicate.
Phase 2. There is a loss of the characteristic odour and taste. The flesh is neutral but has no off-flavours.

TABLE 4.2. **Freshness ratings: Council Regulation (EEC) No. 103/76 OJ No. L20 (28 January 1976)**

Parts of fish inspected	**Criteria**			
	Marks			
	3	2	1	0
	Appearance			
Skin	Bright, iridescent pigmentation, no discoloration	Pigmentation bright but not lustrous	Pigmentation in the process of becoming discoloured and dull	[1] Dull pigmentation
	Aqueous, transparent mucus	Slightly cloudy mucus	Milky mucus	Opaque mucus
Eye	Convex (bulging)	Convex and slightly sunken	Flat	[1] Concave in the centre
	Transparent cornea	Slightly opalescent cornea	Opalescent cornea	Milky cornea
	Black, bright pupil	Black, dull pupil	Opaque pupil	Grey pupil
Gills	Bright colour	Less coloured	Becoming discoloured	[1] Yellowish
	No mucus	Slight traces of clear mucus	Opaque mucus	Milky mucus
Flesh (cut from abdomen)	Bluish, translucent, smooth, shining	Velvety, waxy, dull	Slightly opaque	[1] Opaque
	No change in original colour	Colour slightly changed		
Colour (along vertebral column)	Uncoloured	Slightly pink	Pink	[1] Red

TABLE 4.2. **Freshness ratings** *(cont.)*

Organs	Kidneys and residues of other organs should be bright red, as should the blood inside the aorta	Kidneys and residues of other organs should be dull red; blood becoming discoloured	Kidneys, residues of other organs and blood should be pale red	[1] Kidneys, residues of other organs and blood should be brownish in colour
		Condition		
Flesh	Firm and elastic	Less elastic	Slightly soft (flaccid), less elastic	[1] Soft (flaccid)
	Smooth surface		Waxy (velvety) and dull surface	Scales easily detached from skin, surface rathe wrinkled, inclining to mealy
Vertebral column	Breaks instead of coming away	Sticks	Sticks slightly	[1] Does not stick
Peritoneum	Sticks completely to flesh	Sticks	Sticks slightly	[1] Does not stick
		Smell		
Gills, skin, abdominal cavity	Seaweed	No smell of seaweed or any bad smell	Slightly sour	[1] Sour

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[1] Or in a more advanced state of decay.

Phase 3. There are signs of early spoilage with some off-flavours. In the beginning, the off-flavour may be slightly sour, sickly sweet, fruity or similar to dried fish. In fatty species, rancidity can be detected. During the later stages, cabbage-like, ammoniacal or sulphurous flavours are found.
Phase 4. The fish can be characterized as spoiled and putrid.

At the Lyngby laboratory a numbered scale is used for the panel tasting. The scale is numbered from 0 to 10, 10 indicating absolute freshness, 8 good quality and 6 the neutral (tasteless) fish. The rejection level is 4. Using this scale, the eating quality of cod can be illustrated as shown in Figure 4.2.

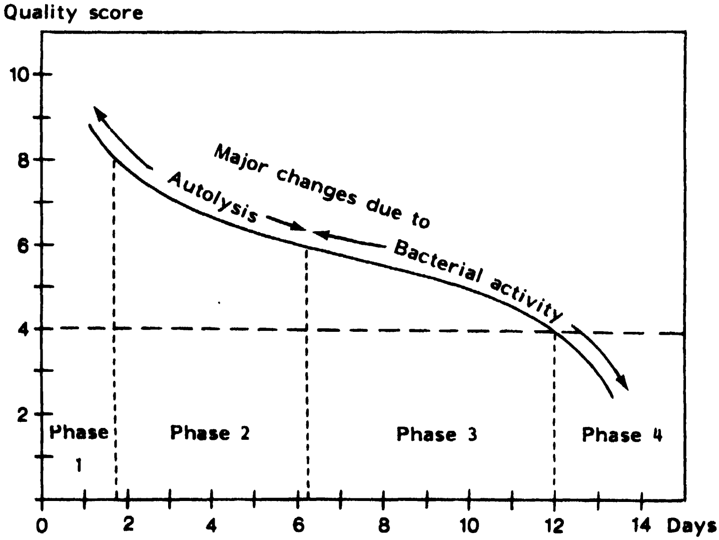


Figure 4.2. Changes in the eating quality of iced (0°C) cod (Huss, 1976)

Autolytic changes

At the death of an organism, the normal regulation system ceases to function and the supply of oxygen and energy production stop. The cells start a new series of processes characterized by the breakdown of glycogen (glycolysis) and the degradation of energy-rich compounds.

Muscle enzymes and their activity

The first autolytic processes in the fish muscle tissue involve the carbohydrates and the nucleotides. For a short period, the muscle cells continue the normal physiological processes but soon the production of adenosine triphosphate (ATP) stops. ATP functions as an ubiquitous energy donor in numerous metabolic processes. In the live organism, ATP is formed by the reaction between adenosine diphosphate (ADP) and creatine phosphate, the latter being a reservoir of energy-rich phosphate in the muscle cells. When the reservoir is depleted, the ATP is regenerated from ADP by rephosphorylation during glycolysis. After death, when the regeneration ceases, the ATP is rapidly degraded. At low ATP levels rigor mortis develops.

In general, fish muscles contain a relatively low amount of glycogen compared with mammalian muscle and the final post-mortem pH is consequently higher; this makes fish meat more susceptible to microbial attack. However, there are great variations in the glycogen content of different species; for example, tuna have levels comparable with those found in mammals, and also within the same species. As a rule, well-rested fish contain more glycogen than exhausted fish, well-fed more than starved fish and large more than small fish. Within the fish, glycogen is more concentrated in the dark muscle than in the white muscle. In stressed fish, glycogen is rapidly utilized. Only five minutes of strenuous exercise cause the glycogen level of rainbow trout (*Salmo gairdneri*) to fall from 0.25 to 0.07 percent of the wet weight (Black *et al.*, 1962). It follows that long trawling times and rough handling accelerate the autolytic processes.

According to Tarr (1966), glycogen is degraded either by glycolysis, i.e. following the Embden-Meyerhof pathway, or by direct amylolytic hydrolysis (see Fig. 4.3).

Since no oxygen is provided, glycolysis in the post-mortem muscle tissue proceeds under anaerobic conditions and, as shown in Fig. 4.3, the end-product is lactic acid. The lactate formed lowers the pH. In cod this decrease is usually from pH 7.0 to pH 6.3-6.9. In some species the final pH may be lower: in big mackerel a pH of 5.8-6.0 is often recorded and in tuna (Tomlinson and Geiger, 1963) and halibut (*Hippoglossus hippoglossus*) values of 5.4-5.6 have been recorded. In other fish such as capelin (*Mallotus villosus*) practically no change in pH is observed. The post-mortem lowering of pH causes a decrease in the water-binding capacity of the proteins since it brings them closer to their isoelectric point (see Fig. 3.3 on p. 21).

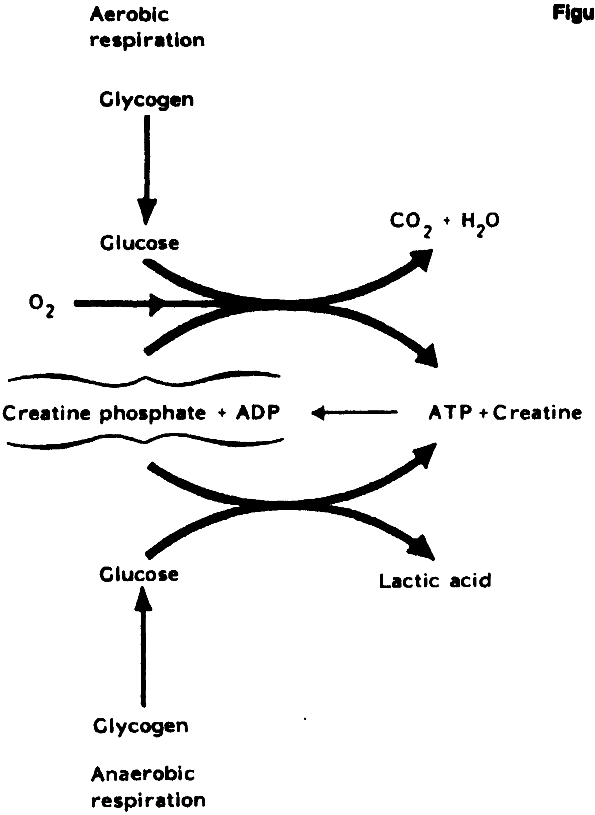
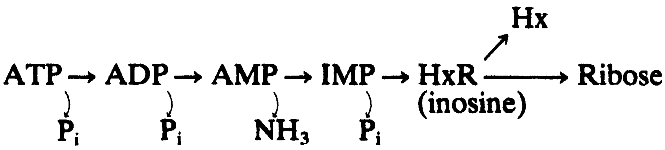


Figure 4.3. Aerobic and anaerobic breakdown of glycogen in fish muscle

ATP is broken down by a series of dephosphorylation and deamination reactions to inosine monophosphate (IMP) which, in turn, is degraded to hypoxanthine (Hx) and ribose:



$$\mathrm{ATP} \underset{P_i}{\rightarrow} \mathrm{ADP} \underset{P_i}{\rightarrow} \mathrm{AMP} \underset{NH_3}{\rightarrow} \mathrm{IMP} \underset{P_i}{\rightarrow} \underset{\text{(inosine)}}{\mathrm{HxR}} \xrightarrow{\nearrow \mathrm{Hx}} \mathrm{Ribose}$$

The above-mentioned autolytic processes proceed in the same manner in all fish but the speed varies tremendously between species. However, for some molluscs it has been reported that the major pathway includes adenosine instead of IMP.

Fraser *et al.* (1967) have followed autolysis in rested cod muscle. The fish were killed after being anaesthetized and were then stored at 0°C. As is shown in Figure 4.4, ATP and glycogen almost disappear before the onset of rigor while IMP and later HxR (inosine) accumulate. When the levels of IMP and HxR start to decrease, the Hx content increases. In trawled fish these changes occur quickly and a minimum pH is often reached within 24 hours of death.

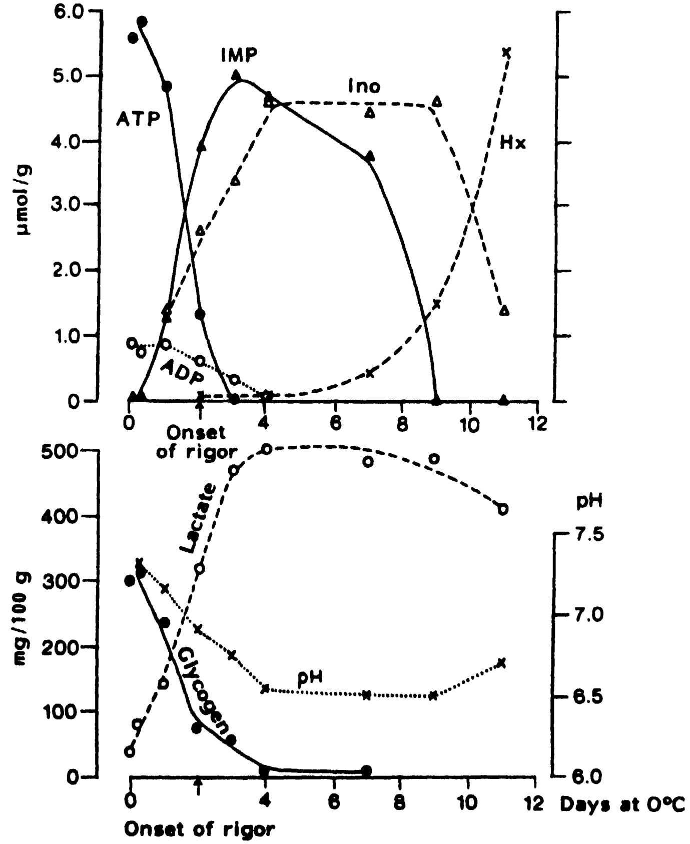


Figure 4.4. (a) Nucleotide degradation in relaxed cod muscle stored at 0°C; (b) the accompanying glycolytic changes (Fraser *et al.*, 1967)

The variations in Hx production of various fish species are illustrated in Figure 4.5 and the changes in Hx, IMP, HxR and organoleptic quality in rainbow trout are shown in Figure 4.6.

Since autolysis always follows the same course in fish, determination of, for example, hypoxanthine has been used as a freshness criterion in some instances, but according to Ehira (1976) this may be misleading if different species are compared. Some species such as horse mackerel (*Trachurus japonicus*) accumulate HxR while others, such as many flatfish, accumulate Hx. A fixed Hx-limit would, therefore, result in flatfish being judged as losing freshness faster than horse mackerel. This contradicts empirical knowledge.

In Japan considerable work has been done in order to establish a more satisfactory expression of freshness and the so-called K-value has

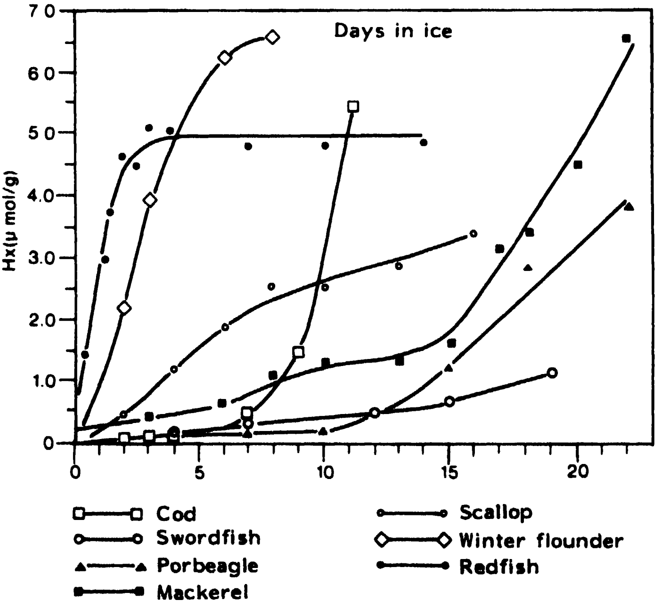


Figure 4.5. Variation in the rate of Hx accumulation of several species during storage in ice: redfish, *Sebastes marinus*; winter flounder, *Pseudopleuronectes americanus*; scallop, *Placopecten magellanicus*; cod, *Gadus morhua*; mackerel, *Scomber scombrus*; porbeagle, *Lamna nasus*; and swordfish, *Xiphias gladius*. Dashed vertical lines indicate the limit of acceptability as judged by taste panel (Fraser *et al.*, 1967)

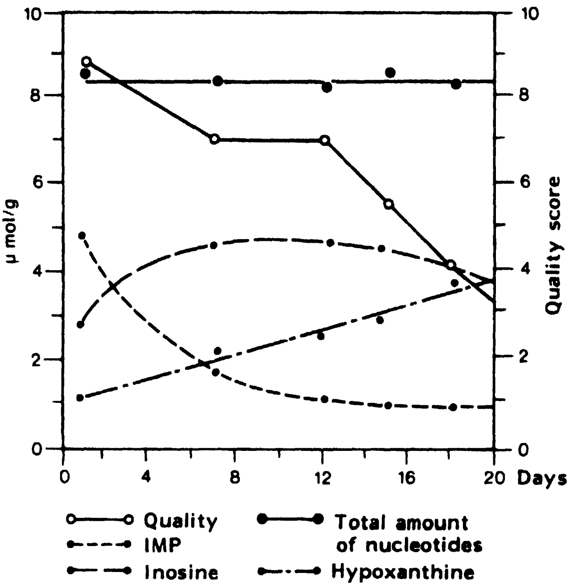


Figure 4.6. Nucleotide degradation and loss of quality in iced rainbow trout (Huss, 1976)

been suggested. This value expresses the relationship between inosine and hypoxanthine and the total amount of ATP-related compounds:

$$K(\%) = \frac{HxR + Hx}{ATP + ADP + AMP + IMP + HxR + Hx}$$

Very fresh fish, therefore, have low K-values, which gradually increase at a species-dependent rate (Fig. 4.7).

The organoleptic importance of the autolytic degradation products is only partially understood. It has been known for a long time in Japan that IMP and other 5'-nucleotides function as strong flavour-enhancers in quite low concentrations, and together with glutamic acid they give rise to a "meaty flavour". Inosine is said to be more or less flavourless, while hypoxanthine has been reported to impart a bitter flavour in spoiling fish (Spinelli, 1965). The loss of flavour in fish flesh is, therefore, attributed to the degradation of IMP.

Free sugars and sugar-nucleotides are of technological importance since they enter into Maillard reactions and thus cause browning during heating.

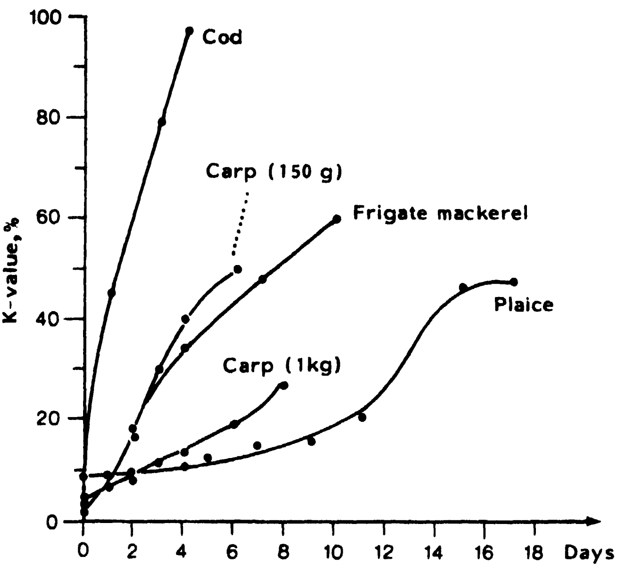


Figure 4.7. Changes in the K-value of instantly killed iced cod (*Gadus morhua macrocephalus*), carp (*Cyprinus carpio*), frigate mackerel (*Auxis* sp.) and Japanese plaice (*Paralichthys olivaceus*) (Ehira, 1976)

The autolytic changes in the proteins are far less pronounced than the changes in nucleotides. Many proteases have been isolated from fish muscle tissue (Reddi *et al.*, 1972; Siebert and Schmitt, 1965). Wojtowicz and Odense (1972) have shown that the main proteases in fish muscle, the cathepsins, have activity levels similar to those of chicken breast muscle. As the latter muscle has rather low autolytic activity, they concluded that the fast autolytic rate of many fish is not due to these enzymes. However, high activity was found in crab and lobster claws and this may be significant in the rapid autolysis of these species.

The muscle cathepsins are hydrolytic enzymes and most of them are located in the lysosomes. Cathepsin D is of major importance since it can initiate the degradation of the endogenous proteins of the cell to peptides. These can then be further degraded by the help of other cathepsins (A, B, C).

According to McLay (1980) and Reddi *et al.* (1972), cathepsin D has optimal activity at pH 4 but the enzyme is able to operate in the range of pH 2-7 (Fig. 4.8b). Somewhat lower values have been reported from Wojtowicz and Odense (1972), who investigated the total catheptic

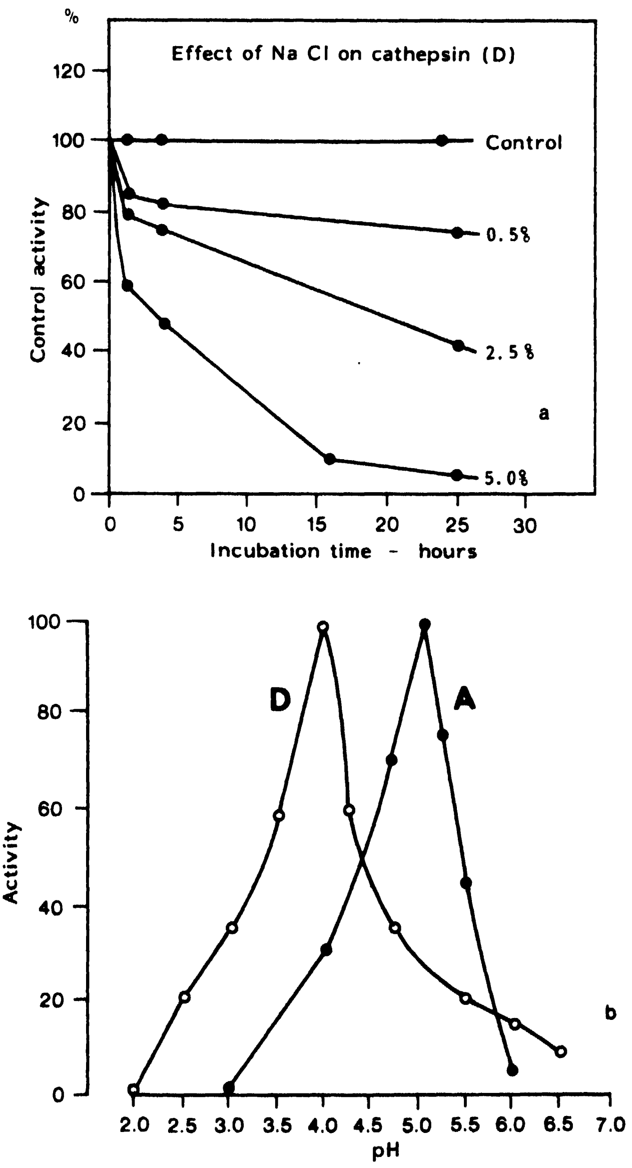


Figure 4.8. Effect of (a) NaCl (b) pH on the activity of cathepsin from fish muscle. The activity is measured after incubation for 30 minutes at 37°C with denatured haemoglobin as substrate ((a) Reddi *et al.*, 1972; (b) McLay, 1980)

activity in fish muscle. The optimal pH of the muscle cathepsins seems to be much lower than the pH found in fish meat and their role during spoilage has not been fully elucidated. It is, however, known that proteolysis due to (cod) muscle enzymes is very limited (Shewan and Jones, 1957) and that proteolysis is not a prerequisite for bacterial spoilage (Lerke *et al.*, 1967). On the other hand, the cathepsins are likely to play a role in the ripening (softening) of marinated fish products which have a rather low pH and low salt content since the activity of the enzymes is greatly inhibited already at 5 percent NaCl (Fig. 4.8a).

Besides cathepsins, some dipeptidases have been found in fish meat (Siebert and Schmitt, 1965; Konagaya, 1978). Interestingly enough, there are no enzymes in the muscle to degrade the sulphur-containing amino-acids as shown by Herbert and Shewan (1976), who could not detect any volatile S-compounds in sterile cod muscle stored for a longer period at high temperatures (Fig. 4.14 on p. 49).

The reduction of TMAO is usually due to bacterial action (Section 4) but in some species an enzyme is present in the muscle tissue which is able to break down TMAO to dimethylamine (DMA) and formaldehyde (FA) (Castell *et al.*, 1973; Mackie and Thomson, 1974):

$$(CH_3)_3NO \rightarrow (CH_3)_2NH + HCHO$$

This process is of limited importance in normally chilled fish since bacteria decompose the TMAO to TMA faster. In extreme cases 2-3 mg of FA and DMA per 100 g muscle have been found in cod stored for two weeks and the TMA content was then 15-20 mg/100 g.

Where bacterial activity is inhibited, the formation of DMA and FA can be of considerable significance as, for example, when cod are frozen. The FA will then cause denaturation, changes in texture and loss of water-binding capacity. Formation of DMA and FA is only a serious problem for cod (gadoids) during frozen storage. However, DMA may accumulate in many fish species during drying and subsequent storage (Hebard *et al.*, 1982).

Digestive enzymes and their activity

It is well known that enzymes from the digestive tract play an important role in the autolysis of whole, uneviscerated fish. During periods of heavy feeding the belly of certain fish (e.g. herring, capelin, sprats and mackerel) is very susceptible to tissue degradation and may burst within a few hours of catch. A thorough understanding of this phenomenon has not yet been reached, but it is known that the connective tissue is weaker if the pH is low and that the post-mortem pH is lowered when the fish are caught during heavy feeding periods (Love, 1980). Furthermore, it is assumed that the production and activity of digestive enzymes is more vigorous during these periods. However, in spite of extensive work, the correlation between extractable proteases and belly bursting has not been very clear (Gildberg, 1982).

The most important digestive proteases are trypsin-like endopeptidases localized in the pyloric caecae, and cathepsin (D) and other pepsin-like enzymes in the stomach wall. These enzymes split the proteins into large-size peptides whereafter different exopeptidases degrade these peptides further (Granroth *et al.*, 1978).

The activity of the digestive enzymes in relation to pH has been the subject of several studies in Norway. When the activity of extractable digestive proteases from capelin is measured during incubation with haemoglobin, the maximum activity is at pH 3 and 9 while it is at neutral pH with glycoprotein extracted from capelin skin (Fig. 4.9). On the other hand, the solubilizing effect of the proteases on muscle tissue

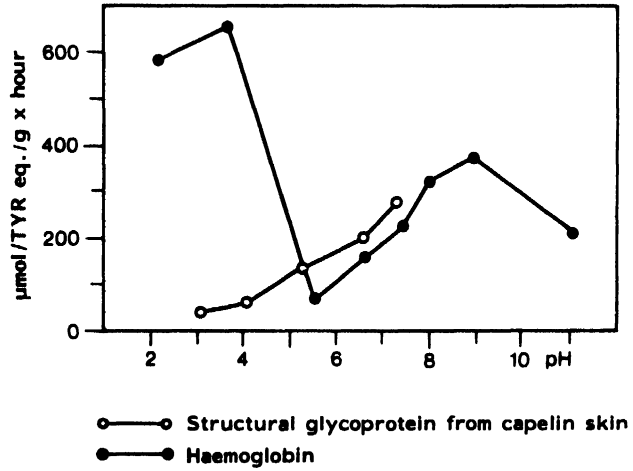


Figure 4.9. Protease activity in homogenized digestive tracts in relation to pH. Activities were determined after incubation for one hour at 25°C. The substrates were haemoglobin and structural glycoprotein from capelin skin (Gildberg and Raa, 1980)

seems to have optima at pH 4 and 7 while the effect on skin shows an optimum at pH 4 (Fig. 4.10). It has been suggested that these differences in protease activities and pH optima depend on whether the substrate is intact tissue containing tissue enzymes inhibitor, collagen, etc. or soluble proteins (Gildberg and Raa, 1980; Hjelmeland and Raa, 1980).

In contrast to cathepsin (D), the digestive enzymes seem to be quite salt-tolerant (Fig. 4.11). In pyloric caecae from herring some carboxy

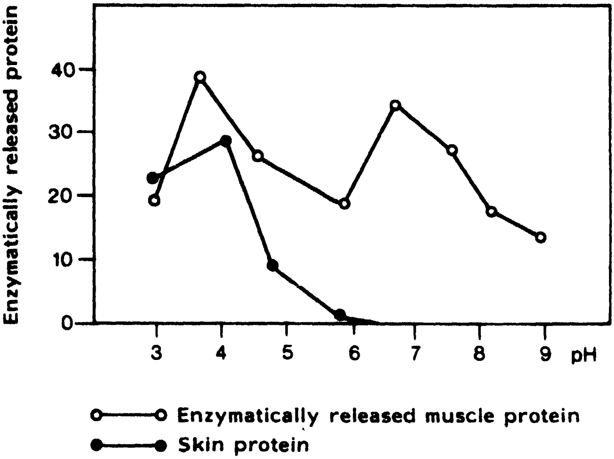


Figure 4.10. Enzymatically released muscle protein and skin protein at different pH values (the difference between incubation with and without the addition of digestive enzymes) (Gildberg and Raa, 1979)

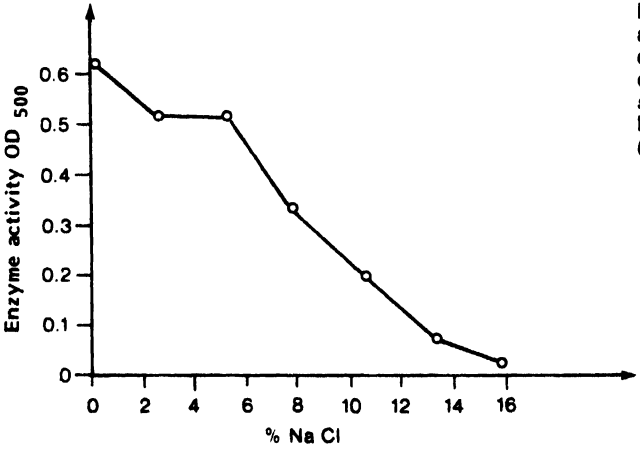


Figure 4.11. The activity of proteolytic enzymes from caecae of sprats (*Sprattus sprattus*) at different NaCl concentrations (37°C) (Marvik, 1976)

peptidases have been found which are even more salt-tolerant, i.e. up to 25 percent NaCl (Granroth *et al.*, 1978). The latter enzymes are likely to play an important part in the ripening of Scandinavian-type salted herring (Knoechel and Huss, 1984) and possibly in some of the Southeast Asian fish sauces. It has often been stated that proper ripening of these products takes place only if some of the digestive tract is left in the fish.

Bacteriological changes

The bacterial flora of live fish

Micro-organisms are found on all the outer surfaces (skin and gills) and in the intestines of live and newly caught fish. A very wide range in their numbers has been reported as shown below:

Skin 10^2-10^7/cm^2
Gills 10^3-10^9/g
Intestines 10^3-10^9/g (Shewan, 1962).

This wide range reflects the effect of the environment. Thus very low numbers (10-100/cm^2 skin) are found in fish taken in clean cold waters (Liston, 1980a; Huss and Eskildsen, 1974) while much higher counts are found in fish from polluted areas or warm tropical waters (Shewan, 1977). It has often been stated that counts in fish intestines are also a reflection of the environment and food intake with nearly sterile conditions being found in non-feeding fish. However, recent work seems to indicate a specific intestinal flora in at least one fish species (*Gadus morhua*) where counts of approximately 10^7/g of a gram-negative *Vibrio*-like organism are regularly found in all fish irrespective of fishing ground, season and food content in the stomach (Larsen *et al.*, 1978). Work in Japan has indicated that the intestinal flora varies with the anatomical features of the digestive tract.

The microbial flora on newly caught fish in temperate waters is mostly reported as being dominated by aerobic or facultatively anaerobic, psychrotrophic, gram-negative rods of the genera *Pseudomonas*, *Alteromonas*, *Moraxella*, *Acinetobacter*, *Flayobacterium*, *Cytophaga* and *Vibrio* (Shewan, 1977). A few analyses on fish from tropical areas are reporting on a preponderance of gram-positive bacteria such as *Micrococcus*, *Bacillus* and coryneforms (Shewan, 1977; Gillespie and Macrae, 1975). However, from the data in his exhaustive review and his own experimental work, Lima dos Santos (1978) has come to the conclusion that gram-positive bacteria do not normally prevail in the microbial flora of tropical fish. What may be more important is the temperature requirement for growth of the bacteria. Shewan (1977) reports on a definitely higher proportion of psychrotrophic bacteria in fish from temperate or cold waters and compares his own data showing only 5 percent of the flora of fish from the North Sea could grow at 37°C compared with approximately 55 percent from fish caught off the Mauritanian coast.

Comparison of data from different sources on the bacterial flora of fish should be carried out very cautiously as pointed out by Lima dos Santos (1978) for a number of reasons. First, it should be noted that bacteria are present in large numbers and that the flora studies are normally limited to a few strains (20-100). This means that only the main groups are likely to be identified and that errors and wrong conclusions may easily be made. Further, it is known that results of bacteriological investigations are greatly affected by the applied methodology. Finally, the taxonomic position of many organisms is still by no means certain.

The flora of freshwater fish is significantly different from that of marine species. Liston (1980a) reports on a high proportion of gram-positive bacteria such as *Streptococcus*, *Micrococcus*, *Bacillus* and coryneforms, while Shewan (1977) points out the presence of the genus *Aeromonas* in all freshwater fish and its absence in marine counterparts. Although the psychrotrophic gram-negative rods seen in marine fish are also dominant in the flora of freshwater fish, the common fish spoilage bacteria, *Alteromonas putrefaciens*, is noticeably absent during the spoilage of Brazilian freshwater fish (Lima dos Santos, 1978). The presence of pathogenic bacteria is discussed in Chapter 7.

Changes in the microflora during storage and spoilage

After an initial lag phase — the length of which depends mainly on temperature — the bacteria on the fish enter into exponential growth and, under aerobic conditions, the total number of bacteria reaches values of 10^8-10^9/g flesh or cm^2 of skin when spoilage is apparent. At low temperatures, the increased number of bacteria is accompanied by qualitative changes, and in marine fish *Pseudomonas* and *Alteromonas* spp. become the dominant genera irrespective of the composition of the initial flora. Shewan (1977) suggests that this is because of the much shorter generation time of these genera at low temperatures. At high ambient temperatures fish spoil very fast (24-36 h), and the flora composition and/or the main spoilage organisms at these temperatures are not known.

Under anaerobic conditions or conditions with low oxygen tension (e.g. vacuum-packaging and storage in chilled seawater), much lower total counts are seen, often reaching a level around 10^6/g fish. However, considerable changes occur in the flora composition as conditions will be favourable for facultative anaerobes able to utilize TMAO or other oxidized compounds in the fish muscle (see sub-section on the following page).

Microbial invasion

In healthy live and freshly caught fish, the muscles are sterile, so bacterial contamination is found only on the outer and inner surfaces of the fish. Formerly, it was often assumed that the bacteria invaded the muscle tissue by way of the vascular tissue or by penetrating the skin. However, histological examinations have shown that in the case of chilled fish only very few bacteria invade the muscle and only at a rather late stage (Shewan and Murray, 1979). Microscopical examination of iced whole cod stored for 12-14 days showed that the fillet as such still contained a very limited number of bacteria. In contrast, the bacteria actually penetrated the flesh via the collagen fibres when the fish were stored at higher temperatures (>+8°C). In chilled fish the main bacteriological activity, therefore, takes place on the surface. Here low molecular weight compounds are attacked and bacterial enzymes diffuse from the surface into the muscle tissue while substrates in the tissue diffuse outwards. Differences in the integument of slime of various species has been proposed as another factor influencing the spoilage rate.

A quick spoiler such as whiting (*Merlangius merlangus*) has a very fragile integument which is very susceptible to damage during handling, while a slow spoiler such as plaice (*Pleuronectes platessa*) has a very robust dermis and epidermis and, furthermore, a thick layer of slime which contains a certain amount of lysozyme (Murray and Fletcher, 1976).

Carbohydrates (e.g. lactate and ribose) and nucleotide fragments are readily available substrates for the bacteria together with the rest of the NPN-fraction.

For aerobic micro-organisms, aerobic oxidation yields much more energy than anaerobic fermentation. Thus, complete oxidation of 1 mole of glucose to 6 moles of CO_2 yields a net production of 36 moles of ATP while fermentation of 1 mole of glucose to 2 moles of lactic acid only gives 2 moles of ATP.

Aerobic:

$$C_6H_{12}O_6 + 6O_2 + 36ADP{+}phosphate \rightarrow 6CO_2 + 42H_2O + 36ATP$$

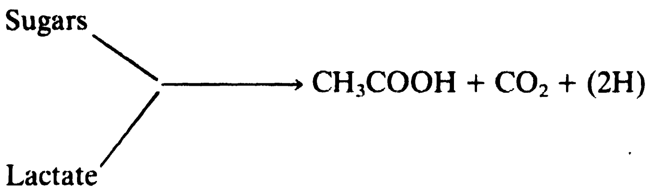
Anaerobic:

$$C_6H_{12}O_6 + 2ADP{+}phosphate \rightarrow 2CH_3CHOHCOOH + 2ATP + 2H_2O$$

Accordingly, the initial bacterial growth under aerobic conditions will primarily be the growth of aerobic organisms using carbohydrates and lactate as energy-yielding substrates, oxygen as hydrogen acceptor with the formation of CO_2 and H_2O as end products.

The growth of aerobic organisms results in the formation of partly anaerobic microclimates on the surface of the fish. Under these conditions, facultatively anaerobic bacteria are favoured. However, for a number of TMAO-reducing bacteria, including some bacteria which are usually classified as obligate aerobes, the presence of TMAO enables these organisms to grow rapidly despite the anaerobic conditions.

The metabolism during TMAO reduction has been investigated recently both in facultative anaerobes such as *E. coli* (Sakaguchi *et al.*, 1980) and *Proteus* sp. (Stenberg *et al.*, 1982) as well as for non-fermentative *Alteromonas* spp. (Easter *et al.*, 1983; Ringø *et al.*, 1984). It now seems certain that in many bacteria TMAO reduction is linked to energy conservation by a respiratory mechanism. During anoxic growth the electrons are sent through the electron transport chain with TMAO as the final electron acceptor and the liberated energy is used for the formation of energy-rich phosphates. In fermentative organisms such as *Proteus*, the catabolic processes are mainly based on fermentative pathways during anaerobic respiration with acetate as a main product:



$$\left.\begin{array}{l}\text{Sugars}\\ \\ \text{Lactate}\end{array}\right\rangle \longrightarrow CH_3COOH + CO_2 + (2H)$$

$$(CH_3)_3NO + (2H) \longrightarrow (CH_3)_3N + H_2O$$

(Kjosbakken and Larsen, 1974)

This mechanism is illustrated in Figure 4.12, which shows that lactate is only used as a substrate when TMAO is present.

Non-fermentative bacteria such as *Alteromonas putrefaciens* retain an aerobic type of metabolism where the amino-acids are completely oxidized to CO_2 while lactate gives rise to some acetate formation. In cold-stored sterile cod muscle the TMAO is not reduced, as shown in Figure 4.13, and it follows that bacterial enzymes are necessary for the reduction. (It should be noted, however, that both the digestive system

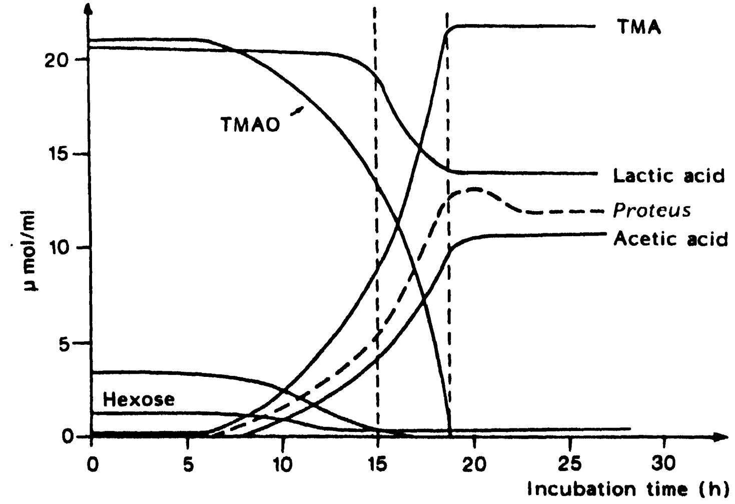


Figure 4.12. Chemical changes in herring extract during anaerobic growth of *Proteus* sp. (Olafsen *et al.*, 1971)

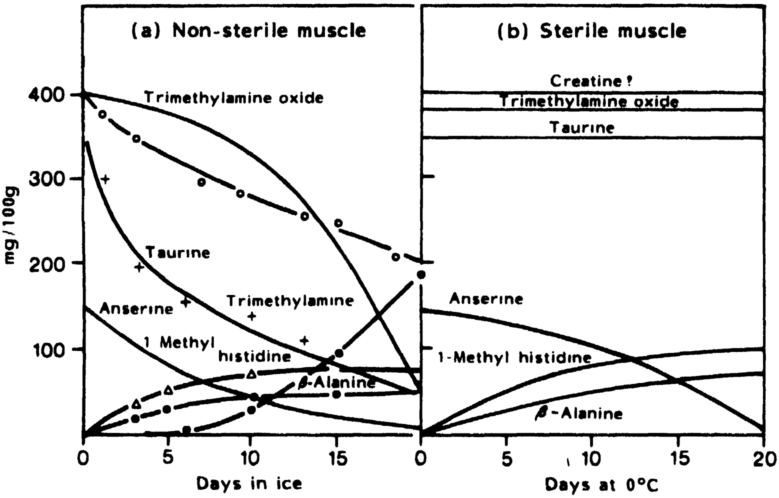


Figure 4.13. Changes in the nitrogenous extractives in (a) spoiling, and (b) autolysing cod muscle (Shewan, 1962)

and the kidney tissue seem to contain a TMAO reductase, which may play a role in minced products while the muscle tissues, as mentioned earlier, contain a reductase which may be important during frozen storage.) The end-product, TMA, has a typical unpleasant "fishy" odour.

TMA constitutes most of the so-called total volatile bases, TVB, above the rejection level found in cold-stored fish. Later during the storage period some ammonia, NH_3, may be formed. A small amount of NH_3 is formed during autolysis but the main part comes from the deamination of the amino-acids. A considerable amount of NH_3 is formed in elasmobranchii during storage because the flesh is rich in urea, which is decomposed by bacteria to CO_2 and NH_3:

$$(NH_2)_2CO + H_2O \xrightarrow{\text{urease}} CO_2 + 2NH_3$$

Shellfish are generally more susceptible to bacterial spoilage than fish. One of the reasons is the higher content of NPN-compounds (Table 3.4). These include a large amount of arginine phosphate, which may constitute up to 1-1.5 percent of the wet weight. Arginine phosphate can be dephosphorylated by autolysis and many bacteria are able to deaminate arginine to ornithine, which can be further decarboxylated to putrescine.

Generally, many of the odours connected with spoilage are the degradation products of amino-acids. The bacterial breakdown of the sulphur-containing amino-acids cysteine and methionine results in the formation of hydrogen sulphide (H_2S), and methyl mercaptane (CH_3SH) and dimethylsulphide ($(CH_3)_2S$), respectively. As shown in Figure 4.14, these compounds are not formed in sterile muscle, but a number of bacteria such as *Alteromonas putrefaciens* are known to be potent producers. These volatile, foul-smelling compounds are organoleptically detectable even at ppb levels, so even minute quantities have a considerable effect on quality.

In an environment with little or no oxygen, the growth of TMAO-reducing bacteria is favoured and TMA production is promoted (Jen-

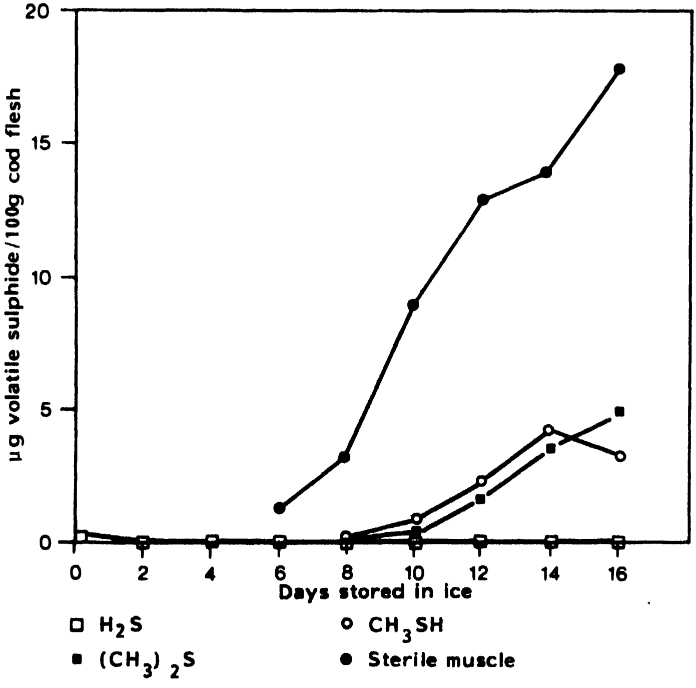


Figure 4.14. Production of H_2S, $(CH_3)_2S$ and CH_3SH in naturally spoiling cod fillets and sterile muscle blocks (Herbert & Shewan, 1976)

sen, 1980; Huss, 1972). A higher TMA content could be taken as an indication of a more rapid spoilage under anaerobic conditions, but this does not seem to be the case in, for example, vacuum-packed fish, as shown by the aforementioned authors. Shaw and Shewan (1968) as well as others have also questioned a direct correlation between TMA content and organoleptic quality of fish.

During the later stages of spoilage, all the TMAO is reduced (usually the fish is rejected organoleptically well before this), and the microbiological milieu changes drastically. According to Norwegian work (Kjosbakken, 1970; Refsnes and Larsen, 1972; Storrø *et al.*, 1977), prolonged anaerobic storage of fish results in vigorous production of NH_3 owing to bacterial degradation of amino-acids and in the accumulation of lower fatty acids such as acetic acid, butyric acid and propionic acid. The strong NH_3-producers were found to be obligate anaerobes belonging to the family Bacteroidaceae genus *Fusobacterium* (Kjosbakken and Larsen, 1974; Storrø *et al.*, 1975, 1977). It is characteristic of these organisms that growth occurs only in spoiled fish extract. Interestingly enough, they seem to have little or no proteolytic activity, thus relying on readily accessible amino-acids for growth.

Specific spoilage bacteria

Even though the total number of bacteria in fresh fish is sometimes very high (see p. 43), many of these bacteria are unimportant during spoilage. Specific spoilage bacteria are characterized by the ability to produce serious off-odours and off-flavours in flesh and these organisms normally constitute only a minor part of the microflora present. During chill storage the percentage of specific spoilage bacteria usually increases (Fig. 4.15). Often these bacteria have a short generation time at chill temperatures, and the TMAO in the fish meat functions as a selective growth-stimulant.

Several methods are employed in order to evaluate the spoilage potential of various micro-organisms. A technique where sterile fish muscle is inoculated with pure cultures of bacteria has been used in the United Kingdom (Shewan, 1977), while Australian researchers have used trypsin-digested sterile homogenate of fish muscle (Gillespie and Macrae, 1975).

At the Technological Laboratory, Danish Ministry of Fisheries, a method has been developed to identify micro-organisms which are able to reduce TMAO and/or produce H_2S from cysteine (Jensen *et al.*,

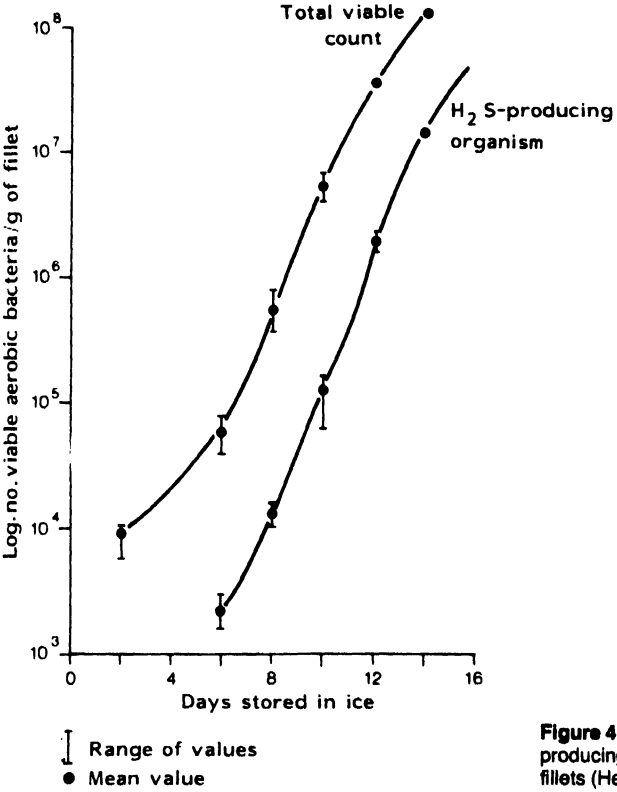


Figure 4.15. Incidence of H_2S-producing organisms in spoiling cod fillets (Herbert *et al.*, 1971)

1980). The ability to produce H_2S from sodium thiosulphate may also be detected directly on a plate count medium (Chapter 5).

The most active specific spoilage bacteria in chilled fish are gram-negative, psychrotrophic rods such as *Alteromonas putrefaciens* and certain *Pseudomonas, Vibrio* and *Aeromonas* spp. (Shewan, 1977).

Rancidity

The most important changes taking place in the lipid fraction are oxidative processes of a purely chemical nature, but enzymatic (microbial or tissue enzymes) degradation may also play a role. With fatty fish in particular, these changes give rise to serious quality problems such as rancid flavours and odours as well as discoloration.

In principle, two types of rancidity are found:

1. Autoxidation, which is a reaction involving oxygen and unsaturated lipid.
2. Lipid autolysis, which is an enzymatic hydrolysis with free fatty acids (FFA) and glycerol as major products.

Autoxidation

There are still many areas of autoxidation which are not sufficiently clarified. However, it is known that the first step leads to the formation of hydroperoxides, that a high number of double bonds makes the lipids more labile and that the process is autocatalytic.

The formation of hydroperoxides involves a free radical chain reaction. The degradation of hydroperoxides is not known in detail, but some of the hydroperoxides give rise to ketones without breaking the carbon chain. Other compounds with rancid odours and flavours are formed after splitting the C-chain. These compounds, after further breakdown, become soluble in water and can thus be rendered accessible to microbial degradation into CO_2 and H_2O.

The free radical chain reaction is easily initiated and once started it is autocatalytic. The free radical reacts with oxygen thus forming a peroxy radical, which then reacts with the substrate to give a hydroperoxide plus another free radical, etc.

The oxidation may be initiated and accelerated by heat, light (especially UV-light) and several organic and inorganic substances. Of the latter, Cu and Fe (often found in salt) are very active catalysts, thus having a strong pro-oxidative effect.

Several natural antioxidants are found. One type is alphatocopherol (Vitamin E), which interferes with the propagation of new radicals and is found in many polyunsaturated vegetable oils such as wheat-germ oil, cottonseed oil and soybean oil. Ascorbic acid (Vitamin C) and, to some extent, carotenoids are also regarded as antioxidants as well as citric acid. The acids prevent oxidation mostly by inactivating the metal ions. Some sulphur compounds have an antioxidative effect by reacting with hydroperoxide in a non-radical process. Antioxidants may also be generated during smoking (wood smoke). These compounds penetrate the outer layers and protect against rancidity.

Rancidity caused by enzymatic activity

This type of lipid degradation involves both lipolytic activity, i.e. lipid hydrolysis, and, in the case of fatty acid decomposition, a lipoxydative activity. The lipid hydrolysis may be brought about by microbial or endogenous lipases. It has been noted in several species that FFA develop more rapidly in ungutted rather than gutted fish, indicating that the viscera contain lipolytic enzymes. The first stage in the reaction is a hydrolysis splitting the triglycerides into glycerol and free fatty acids. During chill storage of eviscerated fish, this hydrolysis is of minor importance, but a considerable amount of FFA may develop during storage of whole, round fish at elevated temperatures (Fig. 4.16).

A clear correlation between the amount of FFA and the degree of rancidity or soap-like flavour has not been established. The further breakdown of the fatty acids is not known in detail. Some microorganisms have lipoxydases which activate the fatty acid chain in a reaction with O_2. The reaction products easily split into aldehydes, ketones, etc., which are typical of rancid flavours.

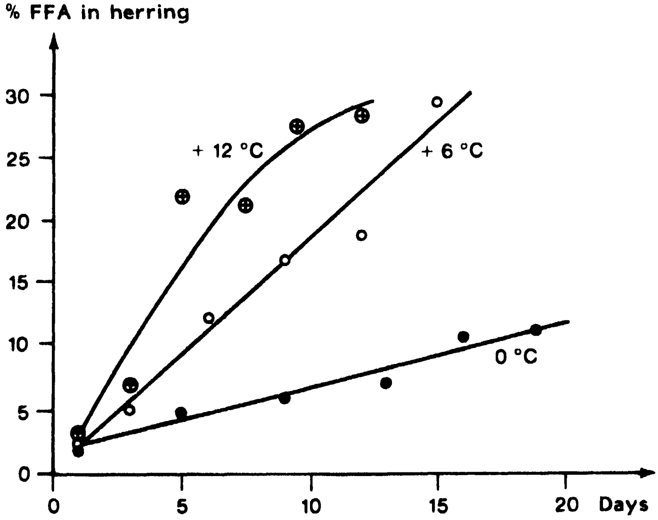


Figure 4.16. The development of free fatty acids in herring stored at different temperatures (Denmark, Ministry of Fisheries)

Physical changes

Changes in pH

As mentioned earlier (see p. 33), the pH of the muscle tissue of live fish is close to neutrality. Due to the post-mortem anaerobic formation of lactic acid, pH decreases usually within the first day of death. During the later post-mortem changes, pH is more or less constant or slightly increased due to the formation of basic compounds.

The initial post-mortem pH varies with species, catching ground and season. The catching method does not seem to influence the final value of the post-mortem pH (Love, 1980). Since fish muscle is not very vascularized, the lactic acid formed through struggling during capture is not readily removed from the muscle. Thus the same amount of lactic acid is accumulated regardless of the amount of muscle stress before death. The seasonal variation in the pH of the meat is to some extent related to the energy reservoir of the fish, e.g. liver glycogen and muscle glycogen. However, much of the glycogen is split hydrolytically into glucose after death (Tarr, 1972), so there is no direct correlation between glycogen content and post-mortem pH. As mentioned previously on p. 33, post-mortem pH may vary considerably (pH 5.4-7.2). It has been shown empirically that some of the differences are species-specific and experimentally that factors such as starvation and refeeding influence the pH, but many questions are still left unanswered. Even though the changes in pH are generally rather small, they have great technological importance. The post-mortem pH is, according to Love (1980), the most significant factor influencing the texture of the meat and the degree of "gaping", i.e. the rupture of the connective tissue (myocommata). One of the reasons for this is that even minor changes in pH drastically affect the properties of the connective tissue. Thus, the mechanical strength at pH 7.1 is four times stronger than that at pH 6.2. Since gaping becomes more pronounced when the fish is frozen, it has been suggested that cod with a pH below 6.7 should not be used for freezing (Love, 1980).

Changes in Eh (oxidation-reduction potential)

In all living forms substrates are being oxidized and thereby yield energy. This process may be split into three types:

1. Reaction with oxygen (hydrolysis + dehydration).
2. Removal of hydrogen (electron + proton).
3. Electron transfer:
   ($Fe^{+2} \rightarrow Fe^{+3} + e^-$).

These reactions have in common the transfer of an electron. A substance is oxidized when it furnishes an electron. Such a substance is called a reducing agent. Other substances accept electrons thereby being reduced. These are called oxidizing agents.

$$De^- \quad + \quad A \rightleftarrows D \quad + \quad Ae \quad + \quad \text{Energy}$$

Electron donor	Electron acceptor	Oxidized state	Reduced state

These electron-transferring systems are called oxidation-reduction systems or just redox systems.

The redox potential, Eh, of the system is the difference between the reduction potential of the reaction and a hydrogen electrode which by definition has a reduction potential of O V. Thus, the redox potential is a measurement of the tendency of the system to furnish and accept electrons. As shown in Figure 4.17, the electrons will move toward systems with higher Eh.

In biological media many simultaneous reactions take place, some of which depend on the presence of enzymes in order to proceed. A

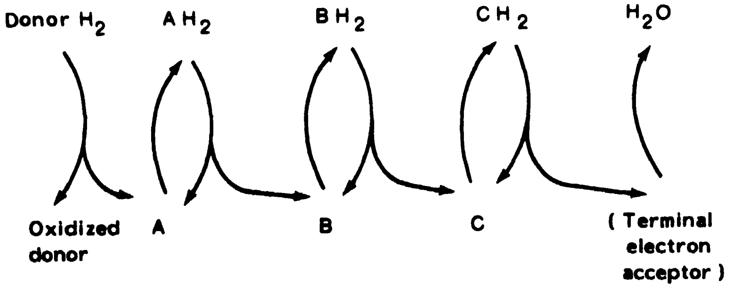


Figure 4.17. Electron transport chain. Eh A<Eh B<Eh C<Eh O_2 (Eh of standard oxygen electrode = +810 mV)

true thermodynamically steady state is, therefore, never obtained, but the measured value yields information about the oxidative status of the system. Usually, the measurements are performed using platinum electrodes and calomel electrodes as a reference. It is of extreme importance to clean the electrodes often and thoroughly as well as to calibrate them in a well-defined medium.

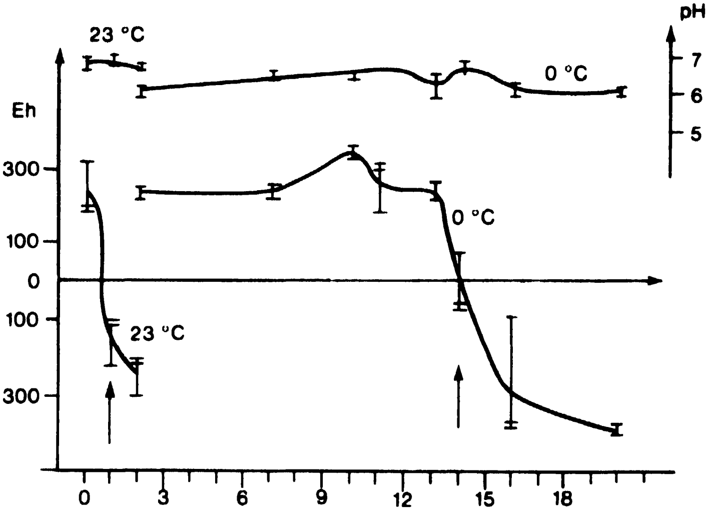
In newly caught fish, Eh always seems to be positive in the muscle tissue (Huss and Larsen, 1979). Around the time that the fish are judged organoleptically unacceptable, Eh decreases rapidly and stays at low, negative values during further spoilage (Figs 4.18 and 4.19).

Judging from the figures, there seems to be a close correlation between Eh and the presence of TMAO. In the samples examined, Eh in the muscle tissues drops almost synchronously with the reduction of TMAO to TMA, whereas Eh in the viscera is negative immediately from capture in accordance with the extremely low content of TMAO in these organs. It has furthermore been shown that in fish products such as salted mackerel (Fig. 4.20), where the TMAO-reducing bacteria are inhibited and the TMAO level is thus more or less constant, Eh does not change significantly and remains positive (Huss and Larsen, 1980).

It should also be noted that in mammals, which in contrast to fish do not contain any TMAO, the redox potential becomes negative very shortly after slaughter (Barnes and Ingram, 1955; Wirth and Leistner, 1970). This, too, is an indication that TMAO is the main factor influencing the post-mortem changes in redox potential in the muscle tissue of marine fish.

Changes in electrical properties

It has long been known empirically that the dielectric properties of biological tissues change markedly after death. The nature of these changes is, however, very complex and at the moment by no means fully understood. It has been suggested that the very rapid decrease in capacitance which occurs within a few hours post-mortem results from a breakdown in the ordered structure of water on the protein surfaces (Jason and Lees, 1971). The more gradual decrease in resistance is attributed to the deterioration of the cell membranes. In very fresh tissue the membranes are only permeable to certain ions and a potential is thus created around each one. The conductance of the cell wall is very low and the resistance high. During storage the membranes



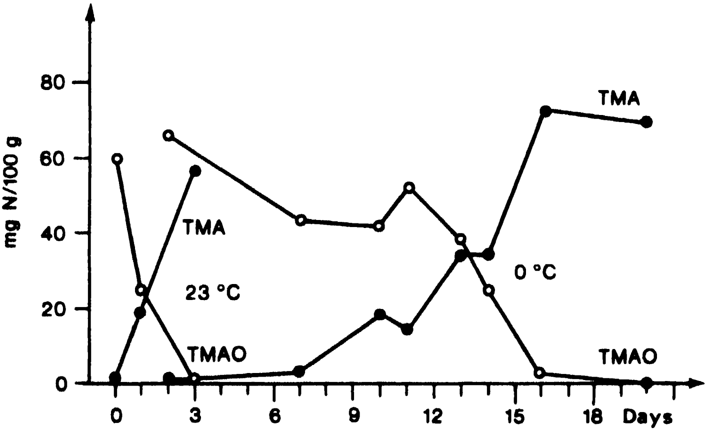


Figure 4.18. Some physical and chemical changes in vacuum-packed cod muscle stored at 0° and 23°C. The arrows indicate the day when the samples were judged unacceptable (Huss and Larsen, 1979)

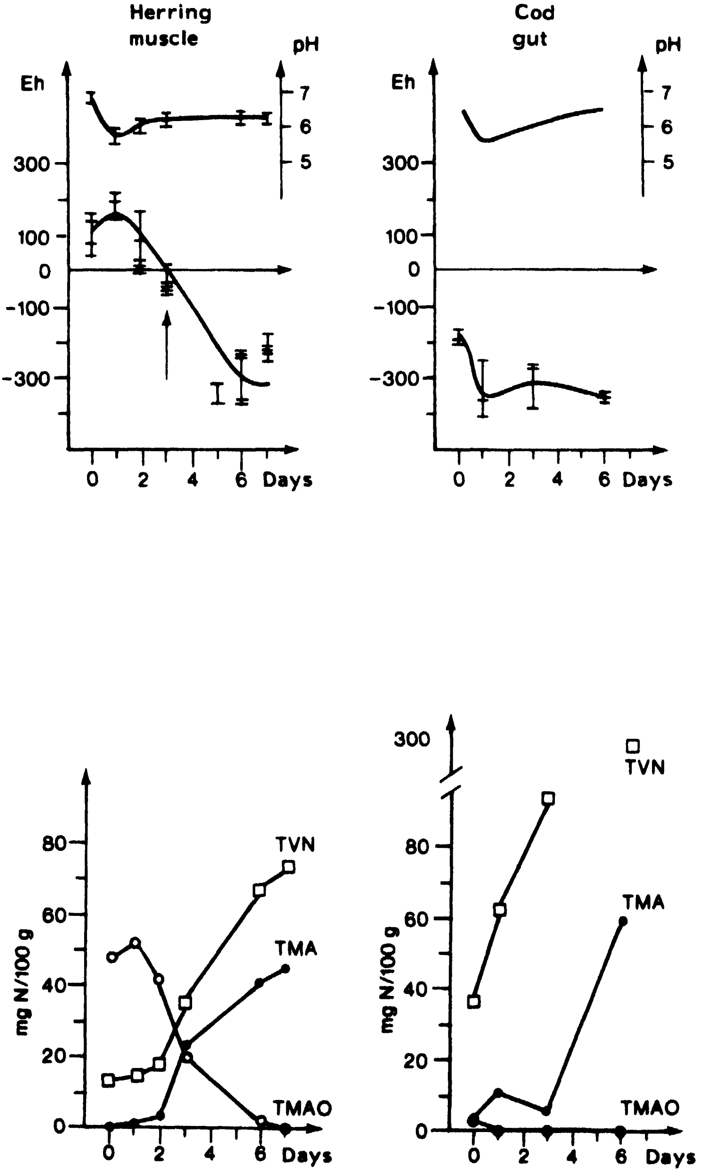


Figure 4.19. Some physical (Eh and pH) and chemical changes in vacuum-packed herring muscle and cod gut stored at +10°C (Huss and Larsen, 1979)

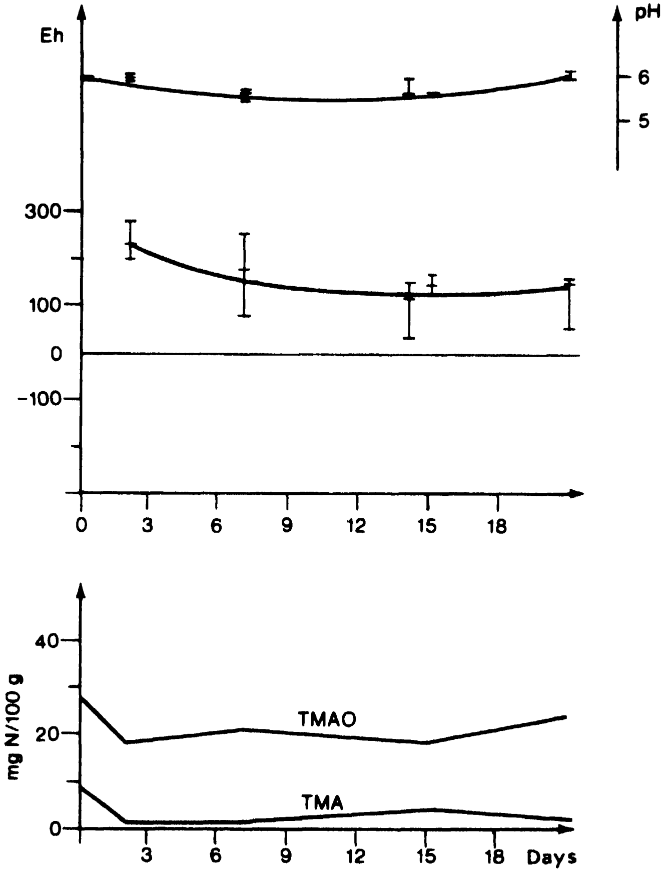


Figure 4.20. Some physical and chemical changes in vacuum-packed salted mackerel (*Scomber scombrus*) with 8 percent NaCl in the aqueous phase stored at +10°C (Huss and Larsen, 1980)

become increasingly permeable to charge carriers, resulting in a lower resistance.

Several factors such as the amount of fat and the temperature of the sample may influence the electrical properties. Furthermore, mechanical damage to the tissues as well as freezing will lead to considerable changes. If the electrical properties of fish tissue are to be used for quality control purposes, all these factors should be considered (Chapter 5).

5. Assessment of fish quality

The word quality is widely used and with many meanings. In the fishing industry the term "quality fish" often relates to expensive species or to the size of the fish. Fish considered by a processor to be of inferior quality may be too small or in too poor a condition for a certain process, resulting in low yields and profit. Most often, however, quality is synonymous with aesthetic appearance and freshness and refers to the degree of spoilage which the fish has undergone. Finally, to government authorities, which are mainly concerned with possible health hazards, good quality means the absence of harmful agents like parasites, chemicals or pathogenic organisms.

A great number of methods have been proposed for assessing the various aspects of fish quality. Some of them have proved to be unsuitable for the purpose, and others are only useful in very specific situations or for a limited number of fish species or products.

Sensory methods

With these methods, the appearance, texture, odour and flavour of the fish samples are evaluated using the human senses. These are the methods which the consumer applies and which give the best idea of freshness or degree of spoilage and general presentation.

The disadvantage of sensory methods is that they are to some degree subjective and depend on the individual judges, their likes and dislikes, prejudices, fatigue and ability to express their sensations when testing a fish product. However, by proper training most of these difficulties can be eliminated.

Much sensory quality assessment is done on an individual basis by one single person, i.e. a fishery officer or fish buyer. His experience will enable him to evaluate fish lots and correct mistakes in handling practices or to correlate quality and price.

In other situations a more objective and descriptive evaluation is needed. In these cases a number of judges may be asked to evaluate the same lot of fish. Their individual assessment may be carried out using a scoring system which allows the results to be pooled, averaged and analysed by ordinary statistics. The number of judges required in this type of analysis is about six, but the use of such a trained panel should be restricted to experimental work, where descriptive profiles of quality changes are collected, or to large companies making branded products.

All institutes of fish technology involved in storage trials need to develop and maintain an experienced taste panel and set aside a room specifically for this purpose. Sensory judgements are influenced by external factors, and the taste panel room should, therefore, be provided with sufficient light, protected from noise and extraneous odours, and preferably provided with shielded booths for each individual judge (see Fig. 5.1).

Most people tend to think in terms of preference or like/dislike when asked to assess fish quality, but such scales are useful only for predicting consumer response to new products, etc. Pair comparisons and triangle tests can be used for pinpointing differences between samples. If quality is to be measured in a more objective way, a scoring system is required.

Figure 5.1. Organoleptic assessment of fish samples at the Technological Laboratory, Danish Ministry of Fisheries

A large number of schemes have been prepared for sensory evaluation. In some cases it is sufficient to have a pass/reject system. Another system, which is in operation in Canada, uses a system where:

Grade I: no spoilage evident
Grade II: signs of early spoilage
Grade III: reject

This system can be further developed as shown in Table 5.1. Thus with increasing experience it is possible to improve the grading by moving from only two classifications (accept/reject) to a 10-point scale.

In Table 5.2 only odour and flavour (raw and cooked) are considered. In some cases appearance and texture of the fish may be equally important and these attributes must be included in the questionnaire or score-sheet given to each judge. An example is shown in Table 5.2. However, many variations are possible and modifications may be required for some species.

TABLE 5.1. **Classification and scoring system for freshness based on odour and flavour of raw and cooked fish**

		Grade		Score
Acceptable	*No* off-odour/flavour	I	Odour/flavour characteristic	10
			of species,	9
			very fresh, seaweedy	8
			Loss of odour/flavour	7
			Neutral	6
	Slight off-odour/flavour	II	Slight off-odours/flavours	5
			such as mousy, garlic,	4
			bready, sour, fruity, rancid	
		Limit of acceptability		
Reject	Severe off-odour/flavour	III	Strong off-odours/flavours	3
			such as stale cabbage, NH_3,	2
			H_2S or sulphides	1

TABLE 5.2. **Model questionnaire. Each judge assesses the samples and marks his findings in the appropriate place and gives an overall quality score. Thus, sample A is given a low score mainly because of discoloration and rancidity, while sample B is rated low owing to deterioration**

Code of sample	**A**	**B**	**C**
Colour			
normal		x	x
discoloration	x		
Odour/flavour			
fresh and characteristic of species			x
loss of odour/flavour			
neutral			
off-odour/flavour non specific			
rancid	x		
old/stale		x	
spoiled			
Texture			
firm	x		x
soft		x	
tough/stringy			
Other comments			
Overall quality score	4	4	10

Name **Date**

Chemical methods

Composition

The chemical composition is an important aspect of fish quality, and it influences both the keeping quality and the technological characteristics of the fish. Since composition may vary considerably with season and catching areas, it is often necessary to make repeated analyses. The *water* content is established by taking a representative sample, drying it in an oven and calculating the weight of the water lost. The *protein* content is assessed by determining the nitrogen content (Kjeldahl method) of the sample and multiplying it by the factor 6.25 (the inverse of N in protein). The sample is analysed for *fat* by extracting the fat from a weighed sample with solvent (e.g. chloroform/methanol), evaporating the solvent and weighing the dry fat residue (Bligh and Dyer, 1959). The *mineral* or ash content is determined by burning off the organic material at a high temperature and weighing the residues from a known initial weight of the sample.

Trimethylamine (TMA)

The most commonly used chemical method for assessing fish quality is the estimation of TMA. This is one of the volatile basic compounds which is found in very low amounts in fresh fish but which accumulates in spoiling marine fish as a result of mainly bacterial reduction of trimethylamine oxide (TMAO). This means that the analysis does not give any information about early autolytic changes or degree of freshness but only about the much later bacterial changes or degree of spoilage.

Figure 5.2 shows the development of TMA in spoiling cod stored in ice (0°C). It has been recommended that good quality cold-water fish contain less than 1.5 mg TMA-N/100 g fish, and 10-15 mg TMA-N/100 g is usually regarded as the limit of acceptability for human consumption (Connell, 1975). It should be noted that these figures apply only to fish in ice. Much larger amounts of TMA are known to be formed during anaerobic storage, i.e. vacuum-packaging or storage in chilled seawater (see p. 46) and in the case of processes such as salting. In these and many other cases there is no correlation between the development of TMA and organoleptic quality as discussed by Ruiter (1971).

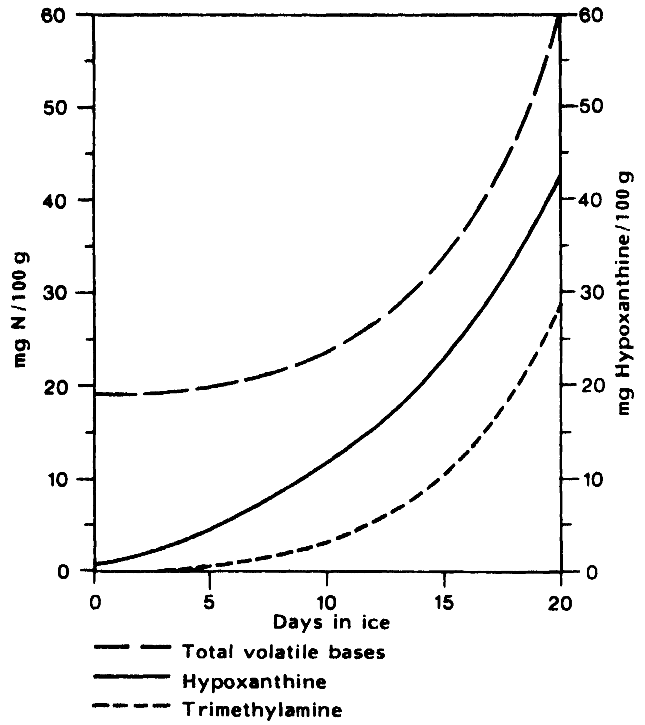


Figure 5.2. The changes in concentration of total volatile bases, hypoxanthine and trimethylamine in iced cod (Connell, 1975)

There are other reasons why TMA is not universally useful as an index of fish quality. Naturally this test cannot be applied to freshwater fish since these species do not contain any TMAO. In marine species, however, the rate of increase in TMA varies considerably from species to species.

In plaice (*Pleuronectes platessa*) and herring (*Clupea harengus*), two important fish species in cold temperate waters, not enough TMA is formed during spoilage to be of analytical use (Connell, 1975). The same may apply to some tropical fish species as reported by Poulter *et al.* (1981) and Amu and Disney (1973). In contrast, it has been demonstrated by Curran *et al.* (1980, 1981a,b) that large amounts of

TMA are formed in some tropical fish species. The latter authors suggest that TMA could be used as an indicator of the incipient point of spoilage but, when these fish are rejected, the level of TMA is well above the limits suggested here for cold-water species.

There are a great number of methods and modifications to them for estimation of TMA, as reviewed by Shewan *et al.* (1971) and Hebard *et al.* (1982). The former authors state that the classical Conway microdiffusion method (Conway and Byrne, 1933) as modified by Beatty and Gibbons (1937) is simple, inexpensive, fairly accurate, reproducible and widely used. However, they also note some disadvantages such as the need for thorough cleaning of the apparatus and accuracy in titration. TMA determination by Dyer's colorimetric method (1945) using picric acid has also been very widely used, but it has undergone a number of modifications as outlined in the review (Hebard *et al.*, 1982).

A number of chromatographic and automated methods and an enzymic method are listed as well as a TMA-specific electrode being mentioned. For detailed procedures for each method, the original publications should be consulted.

Total volatile bases (TVB)

An alternative method to TMA estimation is the measurement of the total amount of volatile basic compounds. The development of TVB is also shown in Figure 5.2, and levels of 30-35 mg TVB-N/100 g in iced cold-water fish are regarded as the limit of acceptability (Connell, 1975).

The method suffers from much the same weaknesses mentioned for TMA. Thus the TVB is low during the edible storage period and, only when the fish is near rejection, does the amount of TVB increase rapidly. In other words, like TMA, a TVB value cannot be used to estimate the degree of freshness in the early stages of storage but only the degree of spoilage in the later stages.

There is also large species-to-species variation in the development of TVB. However, the method has a wider application since it can be used for fish species containing little or no TMAO. Also it is more useful for quality assessment of species where volatile bases other than TMA are formed during spoilage. This is the case for shrimp, octopus and elasmobranchs, where deterioration is characterized by the development of ammonia.

Nucleotide degradation products

Post-mortem desphosphorylation of nucleotides has been studied for the purpose of finding a new index of fish quality (Jones and Murray, 1964).

These processes are part of the early autolytic changes and therefore provide useful information about the degree of freshness during the first part of the storage period. As would be expected, there are great differences between species in the speed of nucleotide degradation (see Figs 4.4 and 4.6), and there are even differences within the same species due to sampling inconsistencies, fish size and environmental factors. Thus it is known that, for example, the Hx content is greater in dark muscle and skin compared with that in white muscle (Fraser Hiltz *et al.*, 1971).

In spite of the above-mentioned factors limiting the use of Hx estimation, it has been shown that it is a useful objective quality test for a number of temperate fish species (Burt, 1977) and for some tropical species (Amu and Disney, 1973; Curran *et al.*, 1981a; 1981b).

Determination of Hx is rather elaborate and requires some skill. A rapid biochemical test was reported by Burt *et al.* (1968), who modified the Hx assay to a colorimetric method employing a redox indicator. A method employing an Hx-sensitive paper strip impregnated with xanthine oxidase, buffer, gelatine and resazurin dye was developed by Jahns *et al.* (1976).

The K-value (see p. 33) is considered by many Japanese workers to be better correlated with fish freshness than Hx content. Fish of the very best quality (edible in the raw state) should according to Ehira (1976) have a K-value of 20 percent or less. Several chromatographic methods are described to measure K-value, such as ion exchange chromatography (Uchiyama and Kakuda, 1983) and reverse-phase ion-pair separation by high-performance liquid chromatography, HPLC (Murray and Thomson, 1983), as well as methods based on the use of enzymes (Uda *et al.*, 1983).

Measurements of oxidative rancidity

The highly labile lipids in fish are susceptible to oxidation. In the early phase peroxides are formed, and these compounds, being odourless and flavourless, can often be detected chemically before any rancidity is organoleptically apparent. The peroxides are eventually further oxidized to aldehydes and ketones, which have a very disagreeable "fishy" or "rancid" odour and taste.

Tests are available to measure both phases of lipid oxidation. The peroxide value (PV) gives a measure of the first stage and the thiobarbituric acid value (TBA) a measure of the second, but unfortunately neither correlates well with sensory assessment of rancidity. Connell (1975) has suggested that if the PV is above 10-20 (units are ml of 0.002 N sodium thiosulphate required to titrate the iodine liberated from 1 g fat extracted from the fish) or the TBA value is above one or two (micromoles of malonaldehyde present in 1 g fat), then the fish will probably smell and taste rancid. PV and TBA value may be estimated by the methods described by Lea (1952) and Vyncke (1975).

Physical methods

Electrical properties

It has long been known that the electrical properties of skin and tissue change after death, and this has been expected to provide a means of measuring post-mortem changes or degree of spoilage. However, many difficulties have been encountered in developing an instrument: for example, species variation; variation within a batch of fish; different instrument readings when fish are damaged, frozen, filleted, bled or not bled; and a poor correlation between instrument reading and sensory analysis (see also p. 56). Most of these problems, it is claimed, are overcome by the recently developed instrument, the GR Torrymeter. However, the instrument is not able to measure quality or freshness of a single fish, but it may find application in grading batches of fish, as shown in Figure 5.3.

pH and Eh

Knowledge about the pH of fish meat may give some valuable information about its condition (see also p. 54). Measurements are carried out using a pH-meter by placing the electrodes (glass-calomel) either directly into the flesh or into a suspension of fish flesh in distilled water.

Measurements of Eh are not carried out routinely, but it is likely that a freshness test can be based on this principle (see p. 54).

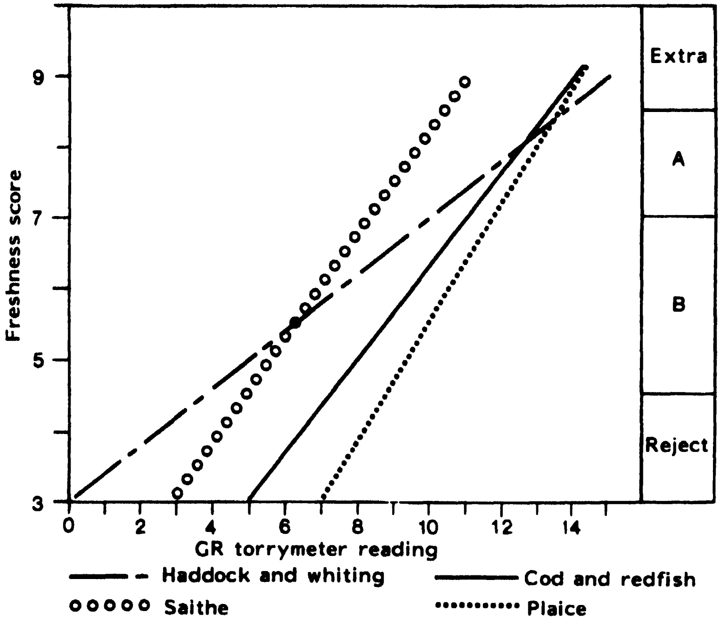


Figure 5.3. Relationship between GR Torrymeter readings of various species of fish and freshness (Cheyne, 1975)

Measuring texture

Texture is a very important property of fish flesh whether it is raw or cooked. A good measure can be obtained by organoleptic testing, by which the texture is judged according to various descriptive terms. For a long time there has been an urgent need for instrumental testing of the texture of fish flesh, so that it can be described objectively in physical terms, preferably corresponding to a system of numbers.

Obtaining reproducible results by instrumental testing of fish muscle texture is, however, extremely difficult. Three of the more promising approaches are briefly described here.

A method for measuring hardness/softness of fish flesh, designated as compressive deformability, has been reported by Johnson *et al.* (1980). An accurately cut fish sample is compressed by a plunger, and the stress-strain curve recorded. A modulus of deformability is calculated from the recorded graph. The results from such measurements may, however, be difficult to interpret.

Another method, measuring the shear force of fish flesh, has been investigated by Dunajski (1980). From this work, it has been concluded that a thin-bladed shear force cell of the Kramer type can be applied.

The third method has been developed by Icelandic researchers Salomonsdottir and Bjarnason (personal communication, 1975). It determines the tearing force generated by placing a sample of fish fillet between two parallel metal plates equipped with 2 cm-long spikes, and sliding the two plates in opposite directions. As the fillet is held between and is penetrated by the spikes, it is torn apart as the plates move. The tear force thus measured is claimed to reflect textural properties of the fish flesh, in particular tissue softness.

If the problem of reproducibility can be minimized to an acceptable level, fish texture can be estimated by any of the methods mentioned. However, regardless of the technique selected, it must be emphasized that numbers obtained by instrumental fish texture measurements must be treated with great caution.

Measuring water-binding capacity

When a certain pressure is exerted on fish muscle, tissue fluids are squeezed out of the muscle. The measured amount of liquid released is used as an estimate of the water-binding capacity, WBC (Hamm, 1972). The WBC varies according to the physical condition of the fish, which is influenced by factors such as seasonal variation, stage of sexual maturation, spawning, etc. In addition, the WBC is low as the fish muscle passes through rigor mortis but increases again afterwards. After prolonged storage there is generally a decline in WBC. Further, there is a correlation between WBC and other physical and chemical properties, e.g. pH and salt content.

A muscle with high WBC is juicy and obtains a high organoleptic score, whereas a muscle with low WBC loses most of its water in the first bite and then appears to be rather dry.

The WBC can be measured by two different principles. The first involves cutting out a tissue sample, placing it on a filter paper, and placing a certain load on top of the sample. The water released is absorbed by the filter paper, and the amount can be determined simply by weighing the filter paper before and after the pressing operation. Several devices have been constructed for mounting the filter paper, sample and top load. The method most frequently used is described by Karmas and Tuerk (1975). The second principle involves pressure exertion obtained by centrifugation. A sample may simply be placed in the bottom of a centrifuge tube, and centrifuged for a certain length of time at a specified speed. The liquid released will be layered on top of the sample. In order to avoid reabsorption of the liquid after centrifugation has stopped, several modifications have been suggested. Among these is the method first suggested by Wierbicki *et al.* (1957), in which the tissue sample rests on a sintered disc positioned halfway down the tube. The tissue fluid released then passes through the disc and is collected in the bottom. Further refinements have been made, and the centrifuge tube set-up illustrated in Figure 5.4 is routinely used at the Technological Laboratory, Danish Ministry of Fisheries.

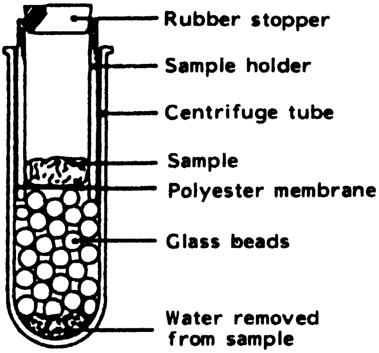


Figure 5.4. Sample holder and centrifuge tube for measuring water-binding capacity in minced fish samples (Eide *et al.*, 1982)

The WBC is usually expressed as tissue water retained as a percentage of the total water in the tissue, which means that the total water content has to be determined separately.

Microbiological methods

In contrast to most other methods, the microbiological procedures do not give any information about freshness or eating quality. The aim of these examinations is to give an impression of the hygienic quality of the fish, the standard of hygiene during handling and processing, and the possible presence of bacteria or organisms of public health significance.

Bacteriological examinations are laborious, time-consuming, costly and require skill in execution and interpretation of the results. It is recommended, therefore, that routine analyses be limited in number and extent. Depending on the actual situation, various microbiological tests may be of interest.

Standard plate count

The standard plate count (SPC) is the number of organisms which develop into clearly visible colonies when the test is carried out under standard conditions and procedures. An SPC of fresh and frozen fish has only a very limited value. If a count is made after systematic sampling and with a thorough knowledge of the handling of the fish before sampling, temperature conditions, packaging, etc., it may give a comparative measure of the overall degree of microbial contamination and the hygiene applied during fish handling and processing (Huss and Eskildsen, 1974). However, it should be noted that there is no correlation between SPC and eating quality or shelf-life of the fish, or between SPC and presence of bacteria of public health significance.

The International Commission on Microbial Specifications for Foods (ICMSF, 1974) recommends that the SPC should be carried out using plate count agar, PCA (Oxoid) with incubation of the plates at 25°C for 3-4 days. The recommended microbiological limits at those conditions attainable in good manufacturing practice (GMP) are in the order of 1-10 million/g fish, but higher counts are quite normal in tropical fish (Shewan, 1977).

An SPC may be carried out replacing plate count agar with iron agar, IA (Jensen, 1980). This medium is rich and normally gives a higher count than PCA. Furthermore, the IA contains sodium thiosulphate and ferricitrate. This means that bacteria able to produce H_2S from sodium thiosulphate form black colonies due to the formation of FeS. These bacteria are regarded as true fish spoilage bacteria since they are also able to reduce TMAO to TMA (unpublished results).

Only small numbers of spoilers are normally found in fresh fish, but the production may increase during storage at low temperatures (see Fig. 4.14). The proportion of total count that consists of black colonies may, therefore, be useful as a measure of the number of days that the fish have been in ice.

Thermotolerant coliform bacteria (E. coli)

Thermotolerant coliform bacteria (faecal coli) are bacteria which ferment lactose and produce gas in lauryl sulphate broth within a period of 48 +/–2 h when incubated at 37 +/–0.2°C, and in addition produce indol in tryptone broth within 24 +/–2 h when incubated at 44 +/–0.2°C. The ICMSF (1974) recommends testing for the presence of this organism as an indicator of post-harvest contamination, particularly a contamination of faecal origin. Most microbiological standards or guidelines recommend a limit of <100 *E. coli*/g fish. Normally the test for *E. coli* is carried out using the most probable number (MPN) technique. It should be noted that the high sensitivity to subzero temperatures makes testing for *E. coli* unsuitable as an indicator of faecal contamination in frozen fish.

Faecal streptococci

Faecal streptococci are gram-positive oblong-oval cocci, occurring in pairs or short chains, which develop wholly or partially pink or dark red colonies of reduced tetrazolium chloride on Slanetz and Bartley's enterococci medium within a period of 48 +/–2 h during incubation at 37 +/–0.2°C. Faecal streptococci are not normally found in fish from uncontaminated waters, and their presence may, therefore, be regarded as an indicator of post-harvest contamination. However, unlike *E. coli*, faecal streptococci are able to multiply in the environment. This means that their presence on fish does not necessarily only indicate a contamination of faecal origin but also generally poor hygiene during handling and processing. Owing to their high resistance to low temperatures, faecal streptococci are useful as an indicator organism in frozen fish and fish products. Most microbial standards recommend a limit of <100 faecal streptococci/g fish.

Staphylococcus aureus

Staphylococcus aureus is an aerobic or facultatively anaerobic gram-positive spherical micro-organism. It is coagulase-positive and ferments glucose. Some strains can produce enterotoxins.

Staphylococcus is not found in the normal microflora on fish. The natural habitat for this organism is the skin and mucous membranes of animal and man; the carrier rate among normal healthy individuals is about 50 percent or more. The presence of *Staphylococcus* on fish is an indication of post-harvest contamination due to poor personal hygiene. The organism is a poor competitor and will not multiply in fish. However, in fish or shellfish products, where the normal flora is reduced or eliminated (i.e. cooked peeled shrimp or crab meat), the presence of staphylococci indicates a potential for food poisoning. ICMSF (1974) suggests a microbiological limit of 1 000 *Staphylococcus aureus*/g.

Salmonella ***spp.***

Salmonella spp. are gram-negative, aerobic or facultatively anaerobic, non-sporing mobile rods. The genus is composed of nearly 2 000 serotypes. Their habitat is the intestines of vertebrates, and most of them are pathogenic to man and/or animals. *Salmonella* should not be found in food or feeds. Testing for *Salmonella* is laborious and costly, and is only recommended for warm-water fish and shellfish harvested in highly polluted areas or in the event of specific cause of concern (e.g. fishmeal).

Vibrio parahaemolyticus

V. parahaemolyticus is an aerobic and facultatively anaerobic, halophilic, often comma-shaped, mobile gram-negative rod. It occurs naturally in fish from warmer waters and cannot, therefore, be excluded from fishery products. The organism is pathogenic to man and has been associated with outbreaks of food poisoning. Analysis for *V. parahaemolyticus* should be applied to products from areas where the incidence of this organism is high and particularly to products which are eaten without prior heat treatment. Recommended microbial limits are <100 *V. parahaemolyticus*/g fish (ICMSF, 1974).

6. Quality and storage life of chilled fish

The effect of fish species, catching method, fishing ground and season

The storage life of different fish species varies considerably, as shown in Table 6.1.

TABLE 6.1. **Storage life of various fish species**

	Shelf-life (days in ice)	Reference
Temperate water:		
Marine species		
white-fleshed lean (cod, haddock, hake)	11-13	a, d
flatfish (sole, plaice)	15-18	a, d
halibut	21	a
fatty fish		
summer herring (fatty)	2- 4	a
winter herring (lean)	12	a
Freshwater sp.		
trout	9-10	d
Tropical water:		
Marine species		
Bahrain (3 species)	13-25	b
Ghana (5 »)	19-22	b
Brunei (3 »)	18-28	b
Sri Lanka (5 »)	20-26	b, e
Seychelles (8 »)	15-24	b
Mexico (6 »)	21-30	b
Hong Kong (2 »)	30-31	b
India (4 »)	7-12	a, c
Freshwater spp.		
Pakistan (2 »)	23-27	b
Uganda (5 »)	20-25	b
East Africa (4 »)	15-28	a, e

Sources: (a) Shewan, 1977; (b) Poulter *et al.*, 1982; (c) Varma *et al.*, 1983; (d) Denmark, Ministry of Fisheries; (e) FAO/DANIDA Workshops, 1983.

In general, it can be stated that flatfish keep longer than round-shaped fish, large fish keep longer than small fish, lean fish keep longer in aerobic storage than fish with a high fat content, and bony fish keep longer than cartilaginous fish. The reasons for these differences are not always clear. The very long rigor period and corresponding low post-mortem pH has been offered as an explanation for the long storage life of halibut (*Hippoglossus hippoglossus*), a very large flat fish. The comparatively short shelf-life of elasmobranchs may be explained by the high content of urea (Table 3.4) and a correspondingly rapid post-mortem development of ammonia. And finally, fish with a high fat content stored in air deteriorate rapidly due to the development of rancidity, a process which at low temperatures is much faster than bacterial spoilage.

With regard to catching methods it is known that, for example, line-caught fish keep longer than trawled, asphyxiated fish because of a slower development of rigor mortis.

In contrast, other differences are not so easy to explain. Various speculations have been offered and there is some evidence to support the hypothesis that a difference in properties of fish slime may account for some of the variations in shelf-life (Shewan, 1977).

Also a great difference between fish species' "freshness lowering rate" as measured by the breakdown of the nucleotides and thus changes in K-value have been recorded (Ehira, 1976). The reason for these differences in autodegradation is not known, but the Japanese studies show no correlation to type of muscle (red or white), type of fish (pelagic or demersal) or habitat temperature.

The data presented in Table 6.1 have caused several workers to suggest that in general tropical fish keep much longer in ice compared with fish from temperate waters (Shewan, 1977; Poulter *et al.*, 1982). The general explanation for this has been that the bacterial flora of fish varies with the environment. Thus psychrotrophic bacteria, which are responsible for spoilage of chilled fish, form an insignificant part of the flora of tropical fish while being the dominant group of bacteria found on fish from temperate waters (Shewan, 1977). However, this has been questioned by Lima dos Santos (1981) in his critical and very exhaustive review listing more than 200 storage trials covering over 100 different tropical fish. He mentions that his data are difficult to analyse, but some general conclusions can be drawn. It is immediately clear that a long (three weeks or more) shelf-life is commonly found in tropical fish but only rarely in fish from temperate water. However, it is equally clear that a storage life of only one or two weeks in ice, which is common for cold-water fish, is also frequently observed in some tropical fish species.

Whatever the reason, it is perhaps not so surprising that the large drop in temperature (up to 30°C) when icing tropical fish has a pronounced effect both on the microbial flora and the autolytic enzymes. It is more surprising that a fish caught in Arctic waters, where the temperature is 0-1°C, still keeps for 10-12 days in ice.

The long shelf-life of tropical fish has considerable practical and commercial implications, as pointed out by Poulter *et al.* (1982). Fish caught close to population centres where only a few days' storage life is necessary need not necessarily be maintained at 0°C. Or, in other cases, where up to three or four weeks are needed for transport and distribution of fish, chilled storage may be preferred to, for instance, frozen storage. Spoilage rates of individual fish species or closely related species are further influenced by fishing ground. Thus it was demonstrated by Huss and Asenjo (1977b) that the spoilage patterns of South American hake (*Merluccius merluccius*) caught in Argentinean, Chilean or Peruvian waters vary greatly. Again the underlying reason for this is not clear, but it may be related to differences in fat content, location of fat or amount of dark muscle.

It has previously been pointed out that pH is a very important parameter in the spoilage pattern of fish (see p. 54). However, this parameter may also show both ground-to-ground and seasonal variations within the same fish species. Very thorough English investigations (Love, 1975) have shown that cod from the Faeroe Islands have a slightly lower pH post-mortem than cod caught near Aberdeen or off the coast of northern Norway. Owing to these differences in pH, cod from the Faeroe Islands in general keep a little longer in ice storage, but they have an increased tendency to "gape" and develop textural defects if they are frozen.

Another important seasonal variation in fish quality is the change related to the reproduction cycle. In fish whose reserves have been severely depleted prior to spawning (such as cod) and with a correspondingly high water content (see p. 12), both quality and nutritional value are reduced.

Off-flavours related to fishing ground

Occasionally, fish with off-flavours are caught, and in certain localities this is a fairly common phenomenon. Several of these off-flavours can be attributed to their feeding on certain organisms such as a planktonic mollusc, *Spiratella helicina*, or the larvae of a *Mytilus* sp. The former gives rise to an off-flavour often described as "mineral oil" or "petrol". It is caused by dimethyl-β-propiothetin, which is converted to dimethylsulphide in the fish (Connell, 1975). The latter is claimed to give a bitter taste in herring. It is furthermore known that cod from Spitsbergen give off an iodine-like odour (Love, 1980). A very well-known off-flavour is the muddy-earthy taint in many freshwater fish, wild as well as cultured varieties. The reason has not been identified unequivocally, but it has been suggested that the taint is caused by the consumption of an *Actinomycetes* sp. with a similar odour (Love, 1980).

Discolorations related to fishing ground and catching method

Customers are usually suspicious of discoloration, and it is considered a serious defect even though the eating quality is not necessarily altered. The most common type of discoloration is the occurrence of blood clots and dark patches in white-fleshed fish. These discolorations are due to rough handling (e.g. long trawling times, throwing the fish, the use of pitchforks) of ungutted and unbled fish. Thus, these discolorations develop during the very early stages of catch handling. Ideally, the fish should be alive when lifted onboard and the risk of colour defects should be reduced by good handling, quick bleeding and chilling.

The fish blood remains fluid for up to 30 minutes if the temperature is kept low, but it clots more rapidly at higher temperatures.

As mentioned earlier on p. 9, it is the muscle contractions during death struggling or rigor mortis which drive the blood out (Huss and Asenjo, 1977a). Thus, it is important to chill the fish and as soon as possible cut open all the major blood vessels before the fish enter rigor mortis. Evisceration of the fish may very well be performed during the same operation since this will open up even more blood vessels.

From time to time the meat of cod or other white-fleshed fish may attain a pinkish colour which has nothing to do with poor bleeding. It is due to either the occurrence of astaxanthin or zeaxanthin in the prey of the fish or metabolic abnormality. Yellow or brownish discolorations are mostly due to oxidation of the remaining blood pigments or the lipids (see p. 51).

Storage temperature

Chilling (0-25°C)

It is well known that both enzymic and microbiological activity are greatly influenced by temperature. However, in the temperature range 0-25°C, microbiological activity is relatively more important, and temperature changes have far greater impact on microbial growth than on enzymic activity (Fig. 6.1).

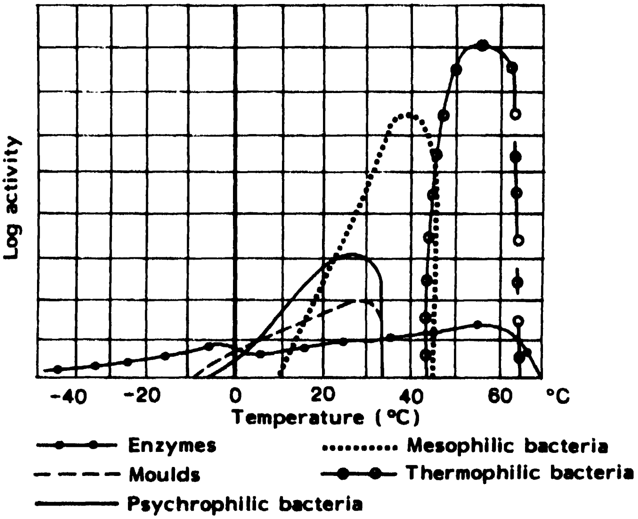


Figure 6.1. Relative enzyme activity and growth rate of bacteria in relation to temperature (Andersen *et al.*, 1965)

As illustrated in Figure 6.2, very minor changes in the temperature range 0-10°C have an enormous effect on the growth of bacteria. Many bacteria are not able to grow well at temperatures below 10°C and even very cold-tolerant bacteria have much longer lag phases and generation times as the temperature approaches 0°C.

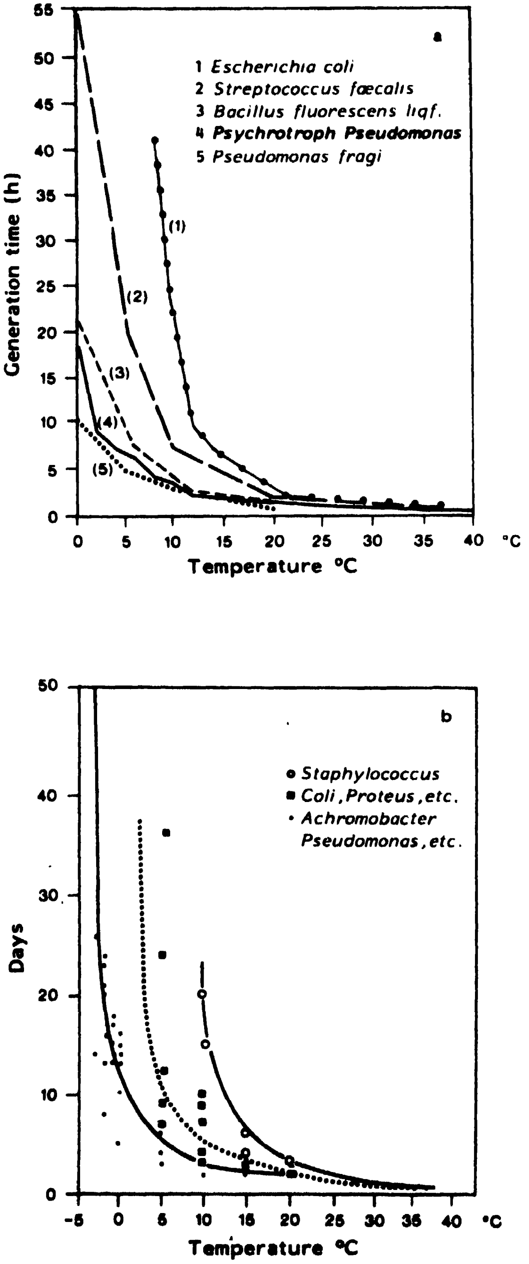


Figure 6.2. Generation time (a) and lag phase (b) of various bacteria in relation to temperature (Elliott and Michener, 1965)

The importance of storing fish at very low temperatures has long been known, as shown in Table 6.2. Similar results have been obtained in Danish investigations, where a rise in temperature from 0° to +5°C results in a 50 percent reduction in shelf-life of both cod fillets and trout packed for retail sale as well as for gutted South American hake, *Merluccius gayi* (Table 6.3).

The relationship between storage life and storage temperature has been thoroughly studied by Australian researchers (Olley and Rat-

TABLE 6.2. **Storage life of cod fillets at different temperatures**

Storage temperature (°C)	Storage life
0	11-12 days
0.5	6-8 »
3	5-6 »
8	2-3 »
10	20-30 hours

Source: Castell, 1949.

TABLE 6.3. **Storage life of various fish at 0° and 4-5°C**

Species	Previous treatment	Storage life (days)		Reference
		0°C	+4-5°C	
Cod	Excellent quality fish, filleted and vacuum-packed in polyethylene	13	7	Huss, 1971
Cod	Medium quality fish (6 days ice), filleted and vacuum-packed in polyethylene	10	5	Huss, 1971
Farmed trout	Gutted, vacuum-packed	18	10	Denmark, Ministry of Fisheries 1971, 1973, 1975
South American hake	Gutted	11	5	Huss and Asenjo, 1977b

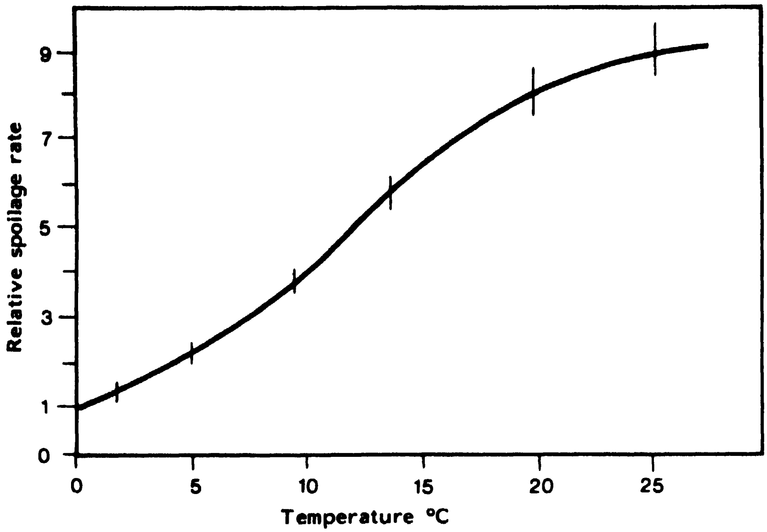


Figure 6.3. Relationship between storage temperature and relative spoilage rate of fish (Olley and Ratkowsky, 1973)

kowsky, 1973). From extensive literature studies and their own results, they constructed the curve shown in Figure 6.3. According to this, the spoilage rate is 2.25 times faster at +5°C than at 0°C. At +10°C, this ratio is 4. It has furthermore been emphasized that the effect of time/ temperature is cumulative. A gutted cod with a keeping time of 12 days at 0°C thus has a considerably shorter storage life if it is exposed to higher temperatures for a short period, as shown in Table 6.4.

TABLE 6.4. **Theoretical storage life of cod after temperature abuse**

Days at		Storage life kept at 0°C
10°C	5°C	
0	0	12
1	0	8 (= 12 − (1 × 4))
0	2	7 1/2 (= 12 − (2 × 2 1/4))
1	1	5 3/4 (= 12 − (1 × 4 + 1 × 2 1/4))

Obviously, temperature is without any exception the most important factor influencing quality and storage life of fresh fish. This statement seems moreover to be valid for all fish species in all countries.

Besides the actual storage temperature, the delay before chilling is of great importance (Fig. 6.4). Thus, it can be observed that if white-fleshed, lean fish enter rigor mortis at temperatures above +17°C, the muscle tissue may be ruptured through severe muscle contractions and weakening of the connective tissue (Love, 1973). The flakes in the fillets separate from one another and this “gaping” ruins the appearance. The fish also becomes difficult to fillet (Table 6.5) and the water-binding capacity decreases.

Rapid chilling is also crucial for the quality of fatty fish. Several experiments have shown that herring and garfish (*Belone belone*) have a significantly reduced storage life if they are exposed to sun and wind for four to six hours before chilling. The reason for the observed rapid quality loss is an oxidation of the lipids resulting in rancid off-flavours. It should be noted, however, that high temperatures are only partly

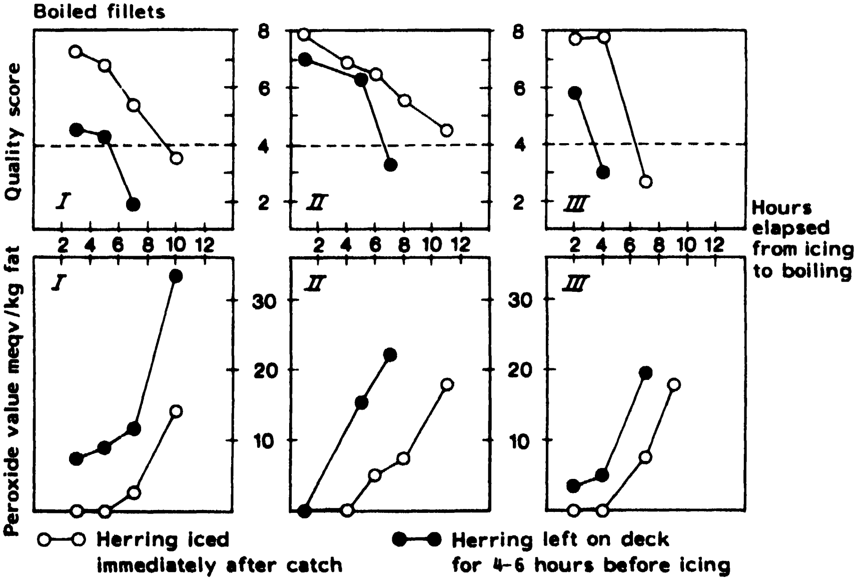


Figure 6.4. Three experiments investigating quality and storage life of herring either iced immediately or four to six hours after catch (Hansen, 1981)

TABLE 6.5. **Fillet yield of gutted cod**

	Iced 1 hour after catch (%)	Iced 6 1/2 hours after catch (%)
Yield of fillet	48.4	46.5
Yield after trimming	43.3	40.4

Source: Hansen, 1981.

responsible for the speed of the oxidation processes. Direct sunlight combined with wind may have been more important in this experiment. As mentioned before (on p. 52), it is difficult to stop the oxidation processes once they have been initiated as they tend to be autocatalytic.

Superchilling or partial freezing (0° to –4°C)

Recognizing the importance of chilling in the storage life of fresh fish, it seems only natural to try even lower temperatures, i.e. from 0 to around –4°C. The process is called superchilling or partial freezing and was studied in the latter part of the 1960s (Merritt, 1965; Partmann, 1965b; Power *et al.*, 1969; Scarlatti, 1965). Recently, Japanese researchers have shown a renewed interest (Uchiyama *et al.*, 1978; Aleman *et al.*, 1982) and they have carried out a number of experiments storing various fish species at –3°C. There is general agreement that partial freezing is able to delay microbial growth for an extended period and the fish remain acceptable for up to four to five weeks depending on the species. However, in the earlier experiments which were mainly based on cod, it was found that the texture of the partially frozen fish is inferior to that of chilled fish partly because of ice formation. In most bony fish freezing starts at –0.8°C, while at –5°C around 62 percent of the water is frozen (Storey, 1980). It was also claimed that the rate of glycolysis and other enzymatic reactions is faster in partially frozen fish (Power *et al.*, 1969) because of the high concentration of enzymes in the remaining unfrozen liquid. These findings are partly contradicted by later experiments (Aleman *et al.*, 1982), which show that the autolytic changes are much slower in partially frozen fish and that glycolysis, too, was delayed compared with iced samples. However, the final amount of lactic acid was slightly higher in the partially frozen fish. According to Uchiyama (1983) it is of extreme importance to keep the temperature constant. This makes partial freezing a rather expensive storage method since it requires special refrigerators.

Hygiene during handling

Onboard handling

Much emphasis has been placed on hygienic handling of the fish right from catch in order to ensure good quality and long storage life. The importance of hygiene during handling onboard has been tested in a series of experiments where various hygienic measures were employed (Huss and Eskildsen, 1974). The quality and storage life of completely aseptically treated fish were compared with fish iced in clean plastic boxes with clean ice and with fish treated badly, i.e. iced in old, dirty wooden boxes. As expected, a considerable difference is found in the bacterial contamination of the three batches (Fig. 6.5). However, a similar difference in the organoleptic quality is detected. During the first week of storage, no difference whatsoever is found. Only during the second week does the initial contamination level become important and the heavily contaminated fish have a reduction in storage life of a few days compared with the other samples. These results are not surprising if it is kept in mind that bacterial activity is normally only important in the later stages of the storage period as illustrated in Figure 4.1.

On the basis of these data, it seems sensible to advocate reasonably hygienic handling procedures including use of clean fish boxes. Very strict hygienic measures do not seem to have great importance. In comparison with the impact of quick and effective chilling, the importance of hygiene is minor.

The above-named observations have influenced the discussion about design of fish boxes. Normally, fish are iced in boxes stacked on top of each other. In this connection it has been argued that fish boxes should have a construction that prevents the ice melt-water from one box draining into the box underneath it. In a system like this, some bacterial contamination of fish in the bottom boxes would be avoided,

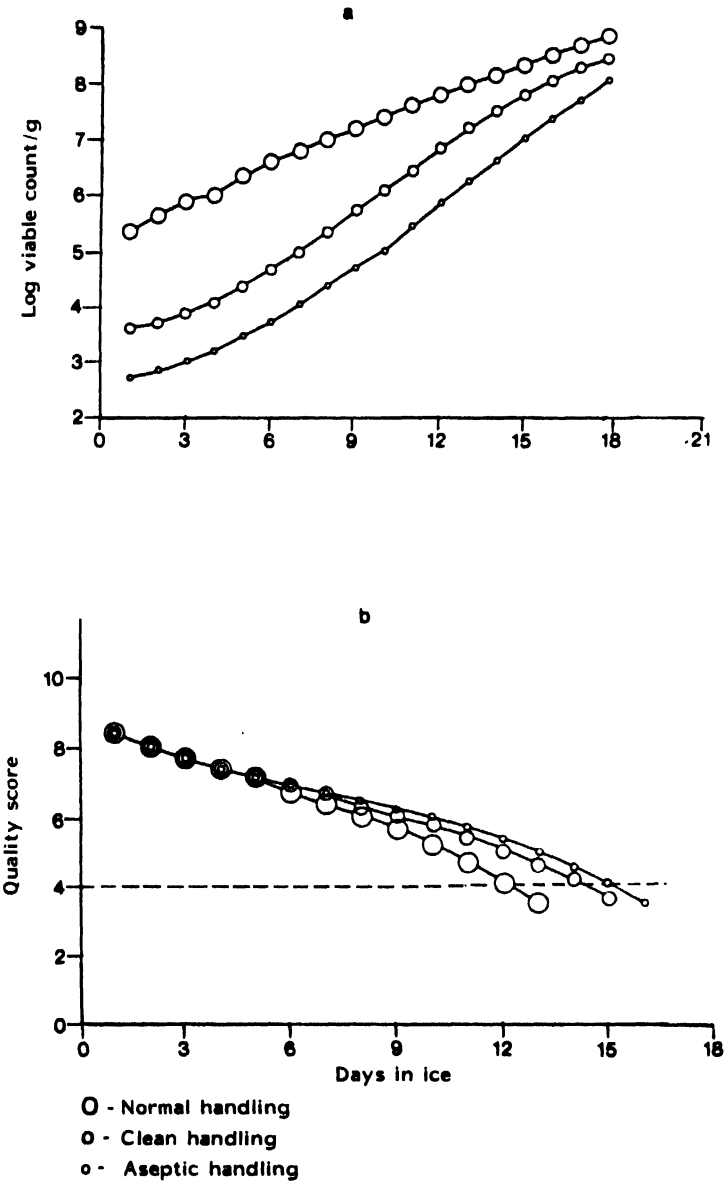


Figure 6.5. Bacterial growth (a) and organoleptic quality (b) of plaice stored at 0°C with initial high, medium and low bacterial counts

as melt-water usually contains a large number of bacteria. However, practical experience as well as experiments (Peters *et al.*, 1974) have shown that this type of contamination is unimportant, and it may be concluded that fish boxes allowing the drainage of melt-water from upper into lower boxes are advantageous because the chilling becomes more effective.

Inhibition or reduction of the naturally occurring microflora

In spite of the relatively minor importance of the naturally occurring microflora in the quality of the fish, much effort has been put into reduction or inhibition of this microflora. Many of these methods are only of academic interest. Among these are (at least until now) the attempts to prolong the storage life by using radioactive irradiation. Doses of 100 000-200 000 rad are sufficient to reduce the number of bacteria and prolong storage life (Hansen, 1968; Connell, 1975), but the process is costly and, to many people, unacceptable in connection with human food. Another method which has been rejected because of concern about public health is treatment with antibiotics incorporated in the ice.

A method that has been used with some success over recent years is treatment with CO_2. CO_2 can be applied either in containers with chilled seawater or as part of a modified atmosphere during distribution or in retail packages (see p. 94).

It should also be mentioned that washing with chlorinated water has been tried as a means of decontaminating fish. However, the amount of chlorine necessary to prolong the storage life creates off-flavours in the fish meat (Huss, 1971). The newly caught fish should be washed in clean seawater without any additives. The purpose of the washing is mainly to remove visible blood and dirt, and it does not cause any significant reduction in the number of bacteria and has no effect on storage life.

Gutting

It is a common experience that the quality and storage life of many fish decrease if they have not been gutted. In feeding periods the fish contain many bacteria in the digestive system and strong digestive enzymes are produced. The latter will be able to cause a violent autolysis post-mortem, which may give rise to strong off-flavours, especially in the belly area, or even cause belly burst. On the other hand, gutting means exposing the belly area and cut surfaces to the air, hereby rendering them more susceptible to oxidation and discoloration. Thus, many factors such as the age of the fish, the species, amount of lipid, catching ground and method, etc. should be taken into consideration before deciding whether gutting is advantageous.

Fatty species

In most cases, small- and medium-sized fatty fish such as herring, sardines and mackerel are not eviscerated immediately after catch. The reason for this is partly that a large number of small fish are caught at the same time and partly because of problems with discoloration and the acceleration of rancidity.

However, problems may arise with ungutted fish during periods of heavy feeding, due to belly burst. As mentioned on p. 40, the reactions leading to belly burst are complex and not fully understood. It is known that the strength of the connective tissue is decreased during these periods and that post-mortem pH is normally lower in well-fed fish and this also weakens the connective tissue (Fig. 6.6). Furthermore, it seems that the type of feed ingested may play an important role in the belly-burst phenomenon.

Lean species

In most North European countries, the gutting of lean species is compulsory. It is based on the assumption that the quality of these species suffers if they are not gutted. In the case of cod, it has been shown that an omission causes a considerable quality loss and a reduction in the storage life of five or six days (Fig. 6.8). After only two days from catch, discoloration of the belly area is visible and the raw fillet acquires an offensive cabbagey odour. As seen in Figure 6.8, these odours are removed to some extent by boiling.

These volatile, foul-smelling compounds are mostly found in the gut and the surrounding area whereas the amount of volatile acids and bases is relatively low in the fillet itself (Fig. 6.9). These chemical parameters are, therefore, not useful for distinguishing between gutted and ungutted fish (Huss and Asenjo, 1976).

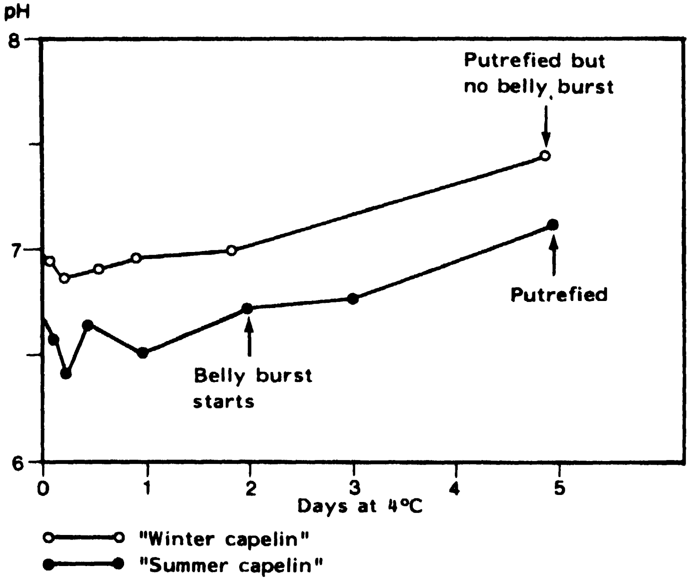


Figure 6.6. pH in winter capelin (○) and summer capelin (●) during storage at +4°C (Gildberg, 1978)

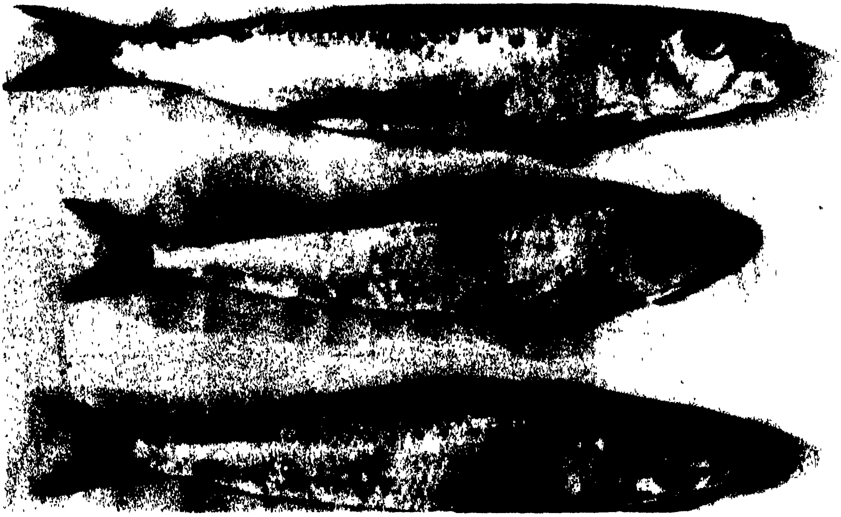


Figure 6.7. Belly burst in Monterrey sardines (*Sardinops sagax caerulea*)

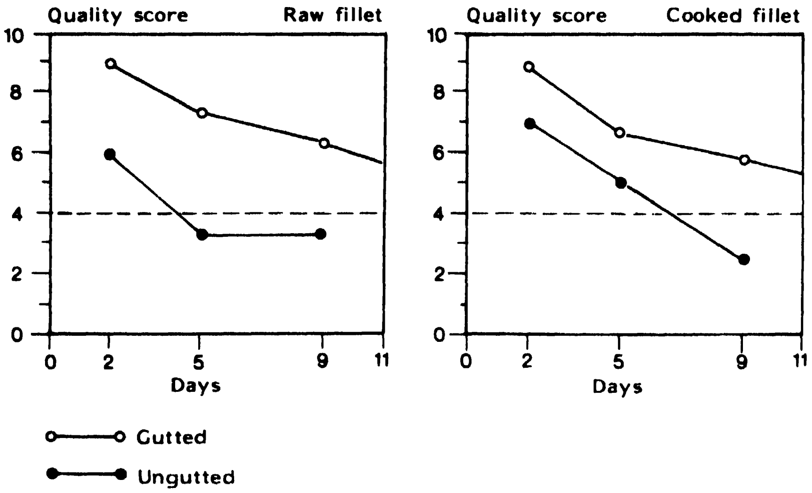


Figure 6.8. Organoleptic quality of raw and boiled fillet, respectively from gutted (○) and ungutted (●) iced cod (Huss, 1976)

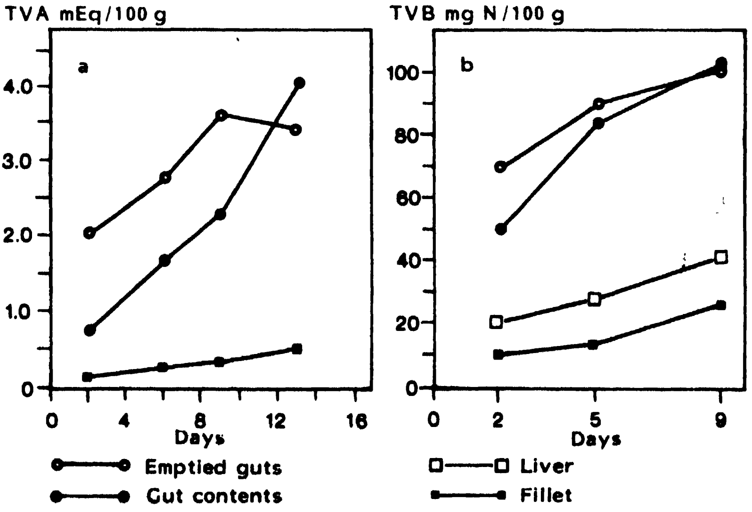


Figure 6.9. Development of (a) volatile acids in iced, ungutted saithe (*Polacchius virens*), and (b) volatile bases in iced, ungutted cod (*Gadus morhua*) (Huss and Asenjo, 1976)

Similar experiments with other cod-like species show a more differentiated picture. In the case of haddock (*Melanogrammus aeglefinus*), whiting (*Merlangus merlangus*), saithe (*Pollachius virens*) and blue whiting (*Micromesistius poutassou*), it is observed that ungutted fish stored at 0°C suffer a quality loss compared with gutted fish, but the degree varies, as illustrated in Figure 6.11. Some off-odours and off-flavours are detected but, as seen in Figure 6.11, ungutted haddock, whiting and saithe are still acceptable as raw material for frozen fillets after nearly one week on ice (Huss and Asenjo, 1976; Denmark, Ministry of Fisheries, 1975). Quite different results are obtained with South American hake (*Merluccius gayi*), where no difference is observed between gutted and ungutted fish (Huss and Asenjo, 1977b).

Figure 6.10. The situation on a South American hake trawler. The fishermen have spent considerable time and effort gutting the fish, where rapid chilling of whole, ungutted fish would have been more beneficial to quality

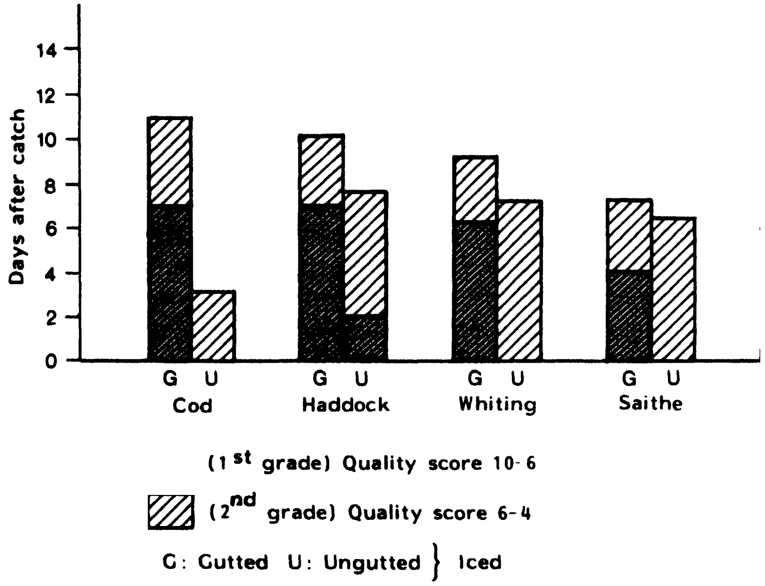


Figure 6.11. Quality and storage life of gutted and ungutted lean fish stored in ice (Huss and Asenjo, 1976)

Composition of gaseous atmosphere

Impact on the microflora

Ordinary air contains approximately 80 percent N_2, 20 percent O_2 and 0.03 percent CO_2. This composition can be changed significantly by one or more methods such as (a) reducing the oxygen tension by, for example, evacuation or replacement of air with nitrogen; (b) increasing the oxygen tension; or (c) increasing the CO_2 tension.

The microbiological processes will be greatly influenced by changes in the gaseous composition. The normal microflora on chilled fish consists mostly of gram-negative aerobic psychrotrophic rods (see p. 43). The growth of these bacteria will be strongly inhibited under anaerobic conditions and the total number of micro-organisms is usually lower on fish stored anaerobically within the period where the fish are edible, as seen in Figure 6.12.

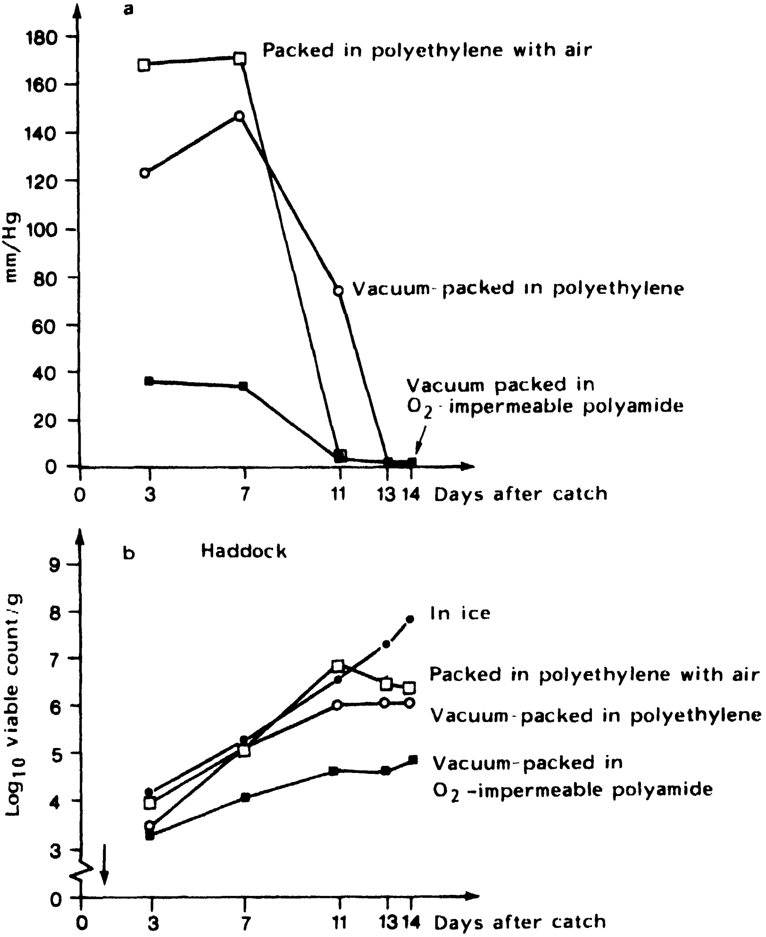


Figure 6.12. The (a) O_2 tension around, and (b) bacterial growth in, haddock packed with various degrees of O_2 exposure (Huss, 1972)

Apart from the quantitative differences, a change in the composition of the microflora will also take place under more or less anaerobic conditions. Certain bacteria, and the potent spoilage bacteria *Alteromonas putrefaciens* in particular, are capable of using TMAO instead of oxygen in their respiration (see p. 43). Because of this advantage these bacteria often become a major part of the microflora during storage. Since they readily attack cysteine in the fish meat, converting it to hydrogen sulphide, they greatly influence the spoilage rate.

CO_2 has long been known to inhibit growth of a number of bacteria (Scott, 1938). Gram-negative, psychrotrophic bacteria, including many common spoilage bacteria, are very susceptible to CO_2 (Gill and Tan, 1980). The details of the inhibition mechanism are not known, but it seems likely that it has an inhibiting effect on certain enzyme systems. Since anaerobic growth of facultative anaerobic bacteria is only slightly affected and *Lactobacillus* spp., which always use a fermentative metabolism, seem to be resistant, it might indicate that the main site of action is connected with oxidative metabolism.

Application of the gas is far more inhibitory to bacteria during the lag phase than during the exponential growth phase (Clark and Lentz, 1969), so it is important to add the CO_2 as early as possible during the process in order to obtain the maximum effect.

Finally, the atmosphere can be modified by increasing the O_2 concentration. Oxygen is known to be toxic in high concentrations for most bacteria due to various mechanisms including inactivation of enzymes, induction of H_2O_2 formation, lipid oxidation and, maybe most important, formation of a free radical O_2^- called superoxide, which may react to form very reactive hydroxyl radicals. However, most aerobic micro-organisms have very good protective mechanisms against O_2 toxicity and increased oxygen concentrations have not been used for practical preservation purposes.

The other preservative principles described in this section have been used during experiments and commercial handling of fish and the following sections summarize some of the experience gained in their use.

Effect of atmosphere on whole fish

Protection against oxygen is of extreme importance when handling fatty fish species such as herring, mackerel, trout, etc. Packing in ice alone provides some protection against light and air. However, O_2 will still be present and the fish will thus turn rancid after some time. If the fish are chilled in water-filled tanks, the environment will soon become anaerobic. Under these conditions oxidation and subsequent rancidity are avoided (Table 6.6).

As mentioned before, however, the anaerobic conditions will favour a certain microflora and the high activity of this microflora is, there-

TABLE 6.6. **Peroxide values (mEq/kg oil) in sprat after one week's storage at 0°C in ice and iced water**

Month of storage	Chilled at 0°C Immediately after catch In iced water	 In ice	 at landing In iced water	 In ice
July	0	27	5	43
September	0	33	5	35

Source: Hansen, 1981.

fore, expected. Practical experiments have shown that the spoilage changes in herring (a fatty fish species) stored in ice and in refrigerated seawater (RSW) are similar during the first four or five days. However, off-flavours related to bacterial activity develop a little earlier in RSW-stored fish, and a disagreeable off-odour develops at a very much earlier stage in the water than in the fish (Smith *et al.*, 1980). In the case of gutted lean species, similar results are obtained (Huss and Asenjo, 1976, 1977b).

Chilling in tanks with refrigerated or chilled seawater, CSW, has other advantages and disadvantages which should be considered. Among the advantages are a more efficient washing, a reduction in weight loss, less mechanical damage and more convenient handling onboard. Among the disadvantages are an increase in salt uptake and, in the case of some fish, a paler appearance and a softer texture.

If CO_2 is added, chilling in tanks becomes much more effective in delaying spoilage. The growth of active spoilage bacteria, such as *Alteromonas* spp. and *Pseudomonas* spp., is inhibited and storage life is prolonged considerably. Thus, it has been found that the storage life of rockfish (*Sebastodes flavidus*) and chum salmon (*Oncorhynchus keta*) is extended by one week (Barnett *et al.*, 1971). In the case of industrial fish, an extension in storage life at 0°C by 9 days (50 percent) is achieved if the fish are chilled in CO_2-saturated (0.3 percent) water while there is almost no effect at 12°C (Olsen, 1977; Mjelde, 1974, 1975). However, the method may be unacceptable for many fish species used for human consumption because of changes in appearance (milky eyes), the development of a slightly sour taste and odour, and textural changes caused by the lowering of the pH.

A side-effect which should be mentioned is the accelerated corrosion rate of metals exposed to high concentrations of CO_2 in the water (Barnett *et al.*, 1971).

A special type of spoilage is observed when fish are stored in contact with old wooden surfaces which are heavily contaminated with an adapted microflora consisting of spoilage bacteria (Maclean and Castell, 1956). Under these conditions the microflora rapidly creates anaerobic conditions at the contact surfaces and starts microbial attack resulting in strong, putrid odours in the affected parts of the fish. These fish are referred to as "stinkers" or as "bilgy" fish.

Pre-packed wet fish

Pre-packed fish may be distributed as whole fish, gutted fish, fillets or parts of fillets. In some countries such as Japan, smaller pieces of fish are often sold in supermarkets in retail packages consisting of flat polystyrene trays loosely wrapped in polyethylene. Since the atmosphere is not changed in any way, spoilage proceeds in the same way as in unpacked fish.

If vacuum-packaging in varying degrees of O_2-impermeable film is used, the O_2 tension is reduced as shown in Figure 6.12. The figure also illustrates that even though evacuation reduces the amount of oxygen within the package, it is the bacterial growth and the corresponding consumption of oxygen which creates the anaerobic conditions. In the case of lean fish the reduction in O_2 tension is of minor importance since the reduction in the total microbial growth is counteracted by an increased production of total volatile bases as shown in Figure 6.13 opposite.

According to these results it is thus concluded that vacuum-packing in polyethylene is recommendable for lean, TMAO-containing fish since it is fairly cheap and easy to pack and chill. In contrast, it was found in similar experiments with fatty fish that vacuum-packaging both improves the quality and extends the shelf-life (see Fig. 6.14 on p. 100).

As indicated in Figure 6.14, the quality of trout and herring stored in air will deteriorate rapidly, and the main reason is the development of rancidity. It is only natural that vacuum-packaging in O_2-impermeable film will prevent rancidity but it is interesting to note that a similar packing in O_2-permeable polyethylene is also able to reduce rancidity and extend storage life. A major contributory cause is the exclusion of

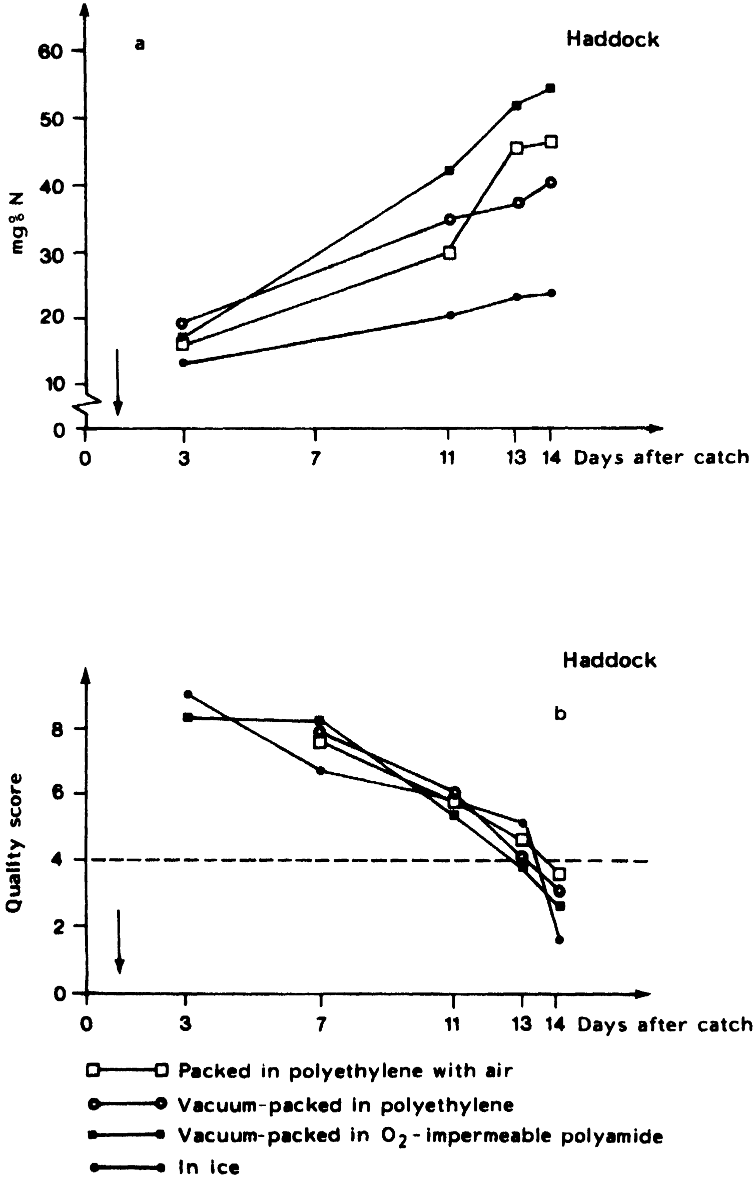


Figure 6.13. The development of (a) total volatile bases in mg percent N, and (b) the taste panel assessment of haddock (Huss, 1972)

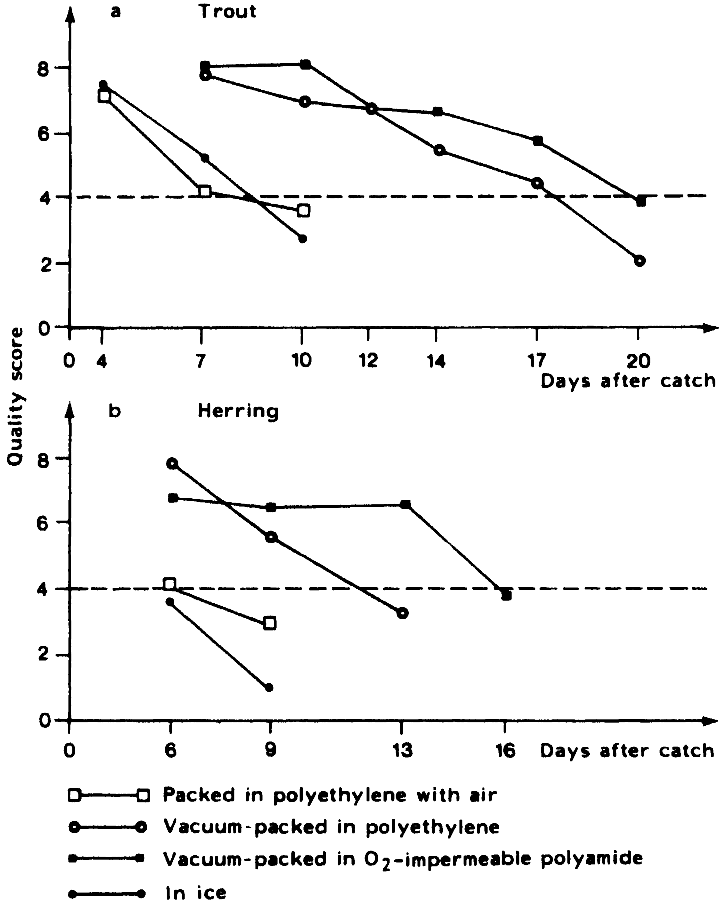


Figure 6.14. Quality and storage life of trout and herring (Hansen, 1972)

air in the belly area due to the belly flaps being squeezed together as shown in Figure 6.15. Rancidity is thus reduced since only the outer, skin-covered surfaces are exposed to air. This is most pronounced in the case of trout since the skin of trout offers more protection than does the herring skin.

Several experiments have been conducted during the last few years

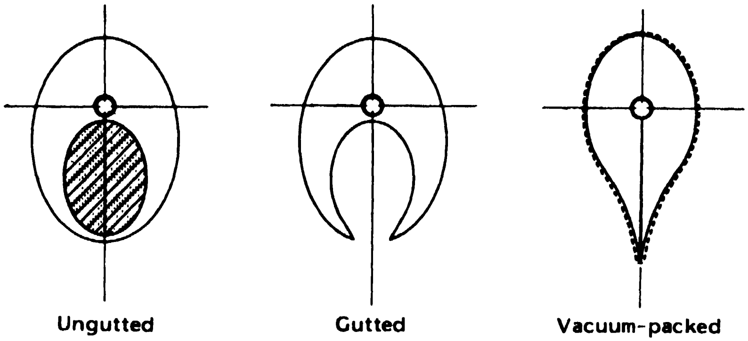


Figure 6.15. A cross-section diagram of trout

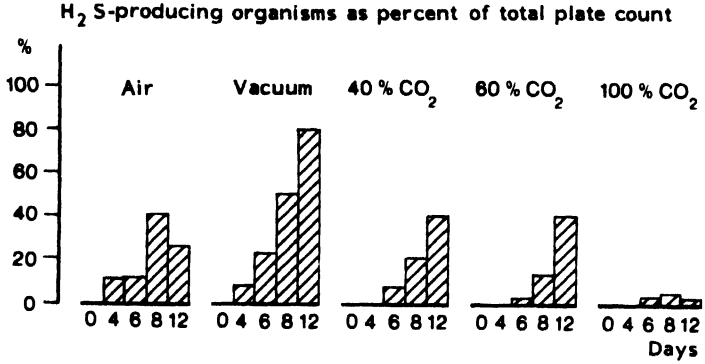


Figure 6.16. H_2S-producing bacteria as percentage of total plate count in cod stored at 2°C (Jensen *et al.*, 1980)

with retail packing in modified atmospheres in order to prolong shelf-life. In most cases a CO_2-enriched gas mixture has been used. In all these investigations CO_2 has been shown to have an inhibitive effect on TMA- and H_2S-producing bacteria while vacuum-packing of TMAO-containing fish often results in a selection of these organisms (Fig. 6.16).

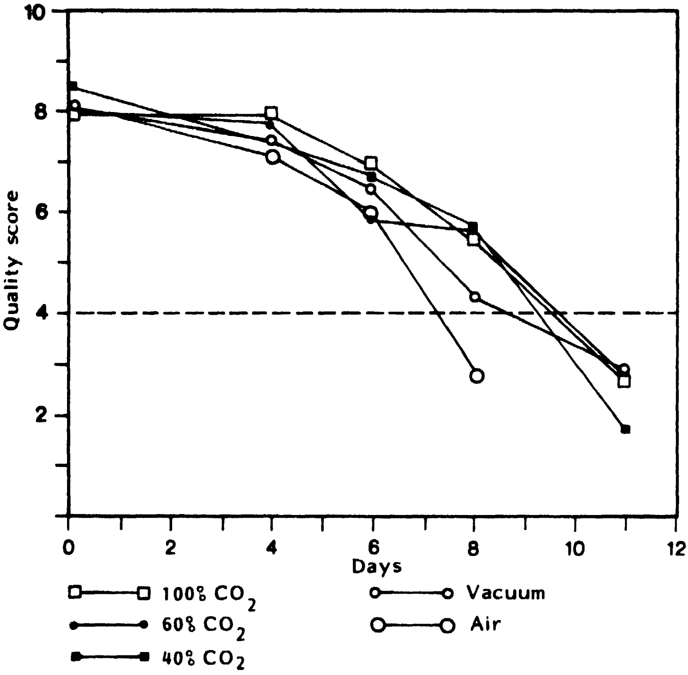


Figure 6.17. Quality and storage life of cod packed in various atmospheres at +2°C (Jensen *et al.*, 1980)

In spite of the quantitative and qualitative differences in the microflora, very little difference is found in the eating quality and storage life (Fig. 6.17) of lean fish. Similar results have been obtained by other researchers (Debevere and Voets, 1971, 1974; Sumner and Gorczyca, 1981; Woyewoda *et al.*, 1984) but some variations are found depending on the permeability of the film and the fish species employed.

7. Public health aspects

Fish and shellfish are capable of transmitting many of the established food-borne infections and intoxications. In addition, there are certain diseases that are most commonly associated with fish, shellfish and the aquatic environment. However, in many countries fish and fishery products are among the safest components of the diet. Geographic position, degree of industrialization, and thus pollution, as well as traditional eating habits are some of the factors determining the health hazards associated with consumption of fish or, for that matter, any other food. Table 7.1 shows that only in Japan are fish a major cause of food poisoning. This is due not only to the very high consumption of fish in Japan but also — and more important — to the custom of eating fish raw.

TABLE 7.1. **Foods associated with outbreaks of food poisoning**

	Fish and shellfish (%)	Meat and poultry (%)	Fruit and vegetables (%)	Other (%)
Canada (1973-75)	5.8	40.3	7.7	46.2
USA (1973-75)	9.3	27.3	3.3	60.1
England and Wales (1973-75)	0.5	12.0	—	87.5
Australia (1967-71)	12.5	43.7	—	43.8
Japan (1968-72)	[1]35.4	?	?	64.6

[1] 34.2% due to *Vibrio parahaemolyticus*.
Source: Liston, 1980b.

Pathogenic bacteria

Fish caught in unpolluted waters are normally free of pathogenic organisms. There are two important exceptions to this: *Clostridium botulinum* and *Vibrio parahaemolyticus* are both known to occur naturally on fish and shellfish from clean waters.

Of the seven types of *Cl. botulinum* known (types A-G), type E is the one most commonly associated with fish and fish products. In marine fish caught in waters around Scandinavia, Alaska and Japan, this is the only type found, and up to 100 percent of the fish may be contaminated. In other parts of the world, types A, B, C, D and F also occur sporadically, as shown in Figure 7.1. In the warm waters of Latin America and Indonesia, type E is noticeably absent, with type C being the most dominant. In both farmed and wild freshwater fish, type E is also the dominant organism. Only in the United Kingdom has a considerable number of types B, C and F been found in farmed trout.

Cl. botulinum is an anaerobic spore-forming organism. It is primarily defined as one species on the basis of the neurotoxin it produces but, culturally, it is a conglomerate of several groups with different metabolic patterns as shown in Table 7.2.

Man can be affected by types A, B, E and F, while type C mainly affects aquatic birds and type D specifically affects cattle. All fresh fish products are excellent media for growth and toxin production by the psychrotrophic non-proteolytic strains of *Clostridium botulinum* (types E, B and F). Increased speed of toxin production is noted at temperatures above + 3.3°C until an optimum temperature of 25-30°C is reached. However, the rate of toxin production is also greatly dependent on the actual sporeload as shown in Table 7.3.

From the data in Table 7.3, it can be seen that within the time necessary for toxin production, raw unprocessed fish will deteriorate organoleptically but not necessarily to the point of rejection. This is particularly the case at elevated temperatures.

TABLE 7.2. **Metabolic groups of *Cl. botulinum***

	I	II	III	IV
Proteolysis	+	–	–	+
Lipolysis	+	+	+	–
Glucose fermentation	+	+	+	–
Amino-acid fermentation	+	–	–	+
Min. growth temperature (°C)	10-12	3-5	10-12	?
Inhibition by NaCl (%)	10	5	3	?
Toxins produced	A,B,F	B,E,F	C_1,C_1,D_1	G

Source: Hobbs, 1983.

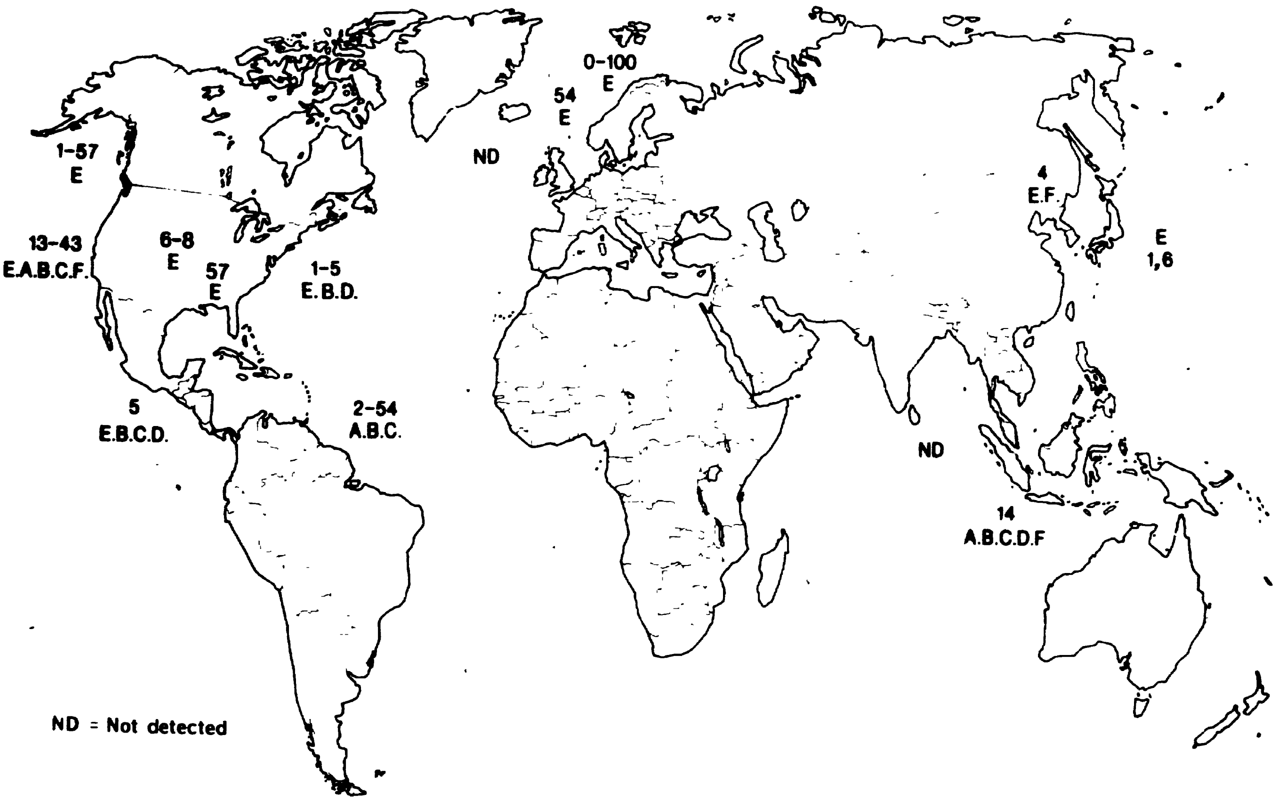


Figure 7.1. Incidence (percentage) of different types of *Cl. botulinum* in fish (Huss. 1981)

TABLE 7.3. **Toxin production in fish flesh and viscera inoculated with *Cl. botulinum* type E spores**

Inoculum		Storage temperature °C	Time to (days)		Reference
Spores/g	Site		Toxin produced	Fish spoiled	
10^1	muscle	5	>36	5-10	(1)
10^3	muscle	5	36		(1)
10^6	muscle	5	15		(1)
10^2	muscle	10	8	2- 5	(2)
10^2	viscera	10	8		(2)
10^1	muscle	15	3	1- 3	(2)
10^1	viscera	15	3		(2)
10^2	muscle	15	1-2		(2)
10^2	viscera	15	1-2		(2)
10^1	muscle	30	1	<1	(2)
10^1	viscera	30	1		(2)

(1) Cann *et al.*, 1965.
(2) Huss, 1981.

Thus evidence points toward fish as being a highly botulinogenic commodity. However, raw unprocessed fish have never caused an outbreak of botulism (Huss, 1981). This is because the botulinum toxin (fortunately) is very heat-labile. At neutral pH, the toxin is completely destroyed within five minutes at 60-80°C (Ohye and Scott, 1957). In a very thorough study, Licciardello *et al.* (1967a and b) concluded that normal cooking procedures (deep-fat frying and pan frying) are sufficient to inactivate any preformed toxin.

In contrast, botulinum toxin is remarkably stable at low pH and in a salty environment (Huss and Rye Petersen, 1980). Processed fish products (e.g. salted, marinated, fermented and cold-smoked) although not being botulinogenic *per se* may, therefore, still become toxic if any preformed toxin is present in the raw material. Strict control with all raw materials used in fish processing is thus essential in any preventive programme.

Vibrio parahaemolyticus is the second pathogenic organism which occurs naturally in fish, particularly those caught in warmer waters. This organism is a mobile gram-negative, halophilic marine organism which grows well on seafood at temperatures above +8°C, but optimum conditions for growth are an alkaline medium containing 2-4 percent NaCl at 37°C. The generation time at optimum conditions is very short (five to ten minutes), and it competes well with other organisms. This means that small numbers of *V. parahaemolyticus*, which are harmless, can grow to large populations capable of causing food poisoning in a few hours at suitable temperatures. The dose required to cause illness has been estimated to be 10^6-10^9 cells. The disease is characterized by diarrhoea, abdominal pain, possibly vomiting, mild fever and headache. The symptoms appear from four to 48 hours after consumption of the food, and recovery is usually uneventful and complete in two to six days. The foods involved are mainly raw or lightly cooked fish products.

V. parahaemolyticus is essentially a coastal and estuarine organism and has only rarely been isolated from fish caught in the open sea. Hobbs (1982) states in his review that *V. parahaemolyticus* has been detected in all coastal zones investigated. Its growth depends not only on temperature but also on the presence of organic nutrients such as those which may derive from fish processing or sewage.

V. parahaemolyticus is sensitive to chill temperatures (0-8°C), as shown in Table 7.4. However, it survives the freezing process and has been isolated from frozen fishery products.

The significance of the possible presence of *V. parahaemolyticus* in the natural environment varies with climate and dietary habits. Thus, in Japan, which has high ambient temperatures in the summer and where there is a habit of eating raw fish, poisoning by this organism is common and accounts for most of the outbreaks reported in Table 7.1.

Fish caught in coastal areas are affected by pollution from sewage or other sources and may carry a number of organisms which are able to

TABLE 7.4. **Survival of *V. parahaemolyticus* in various seafoods stored at 0°C**

Storage time (days)	**Survival** (%)			
	Oyster	**Fish**	**Crab**	**Shrimp**
0	100	100	100	100
2	15	11	35	10
8	5	1	1	10

Source: Liston, 1980b.

cause food poisoning. Typical water-borne (and fish-borne) pathogens are *Salmonella*, *Shigella*, vibrios (*V. cholera*), *Aeromonas hydrophila* and the hepatitis A virus. However, fish and shellfish are not important sources of diseases caused by these organisms.

Parasites

The presence of parasites in fish is very common. The great majority are of no public health significance, but they represent a quality problem since their presence is unaesthetic and causes rejection and complaints from the buyer and/or consumer. Thus in certain geographical areas where infection by parasites is particularly serious, the fish processor has to make arrangements, often at great cost, for the detection and removal of parasites.

Protozoan parasites are common in bony fish, but are without any public health significance. However, infection by *Myxosporidea* has some technological interest since they are known to exercise high extracellular protease activity and thereby cause softening or jellification of the fish flesh (milky spots). This quality defect has been found in South American hake (*Merluccius* sp.). The parasite is difficult to see with the naked eye, but after a few days in ice the fish flesh becomes soft and jelly-like where the parasites are located.

Another type of protozoan parasite is *Henneguya zschokkei,* found in Pacific salmon species (*Oncorhynchus* spp.) (Christensen and Ellemann, 1976). The parasites cause round, grey-white lesions up to 0.5-1 cm in diameter. The lesions are cysts consisting of an outer capsule of connective tissue and a centre of a creamy substance containing numerous spores measuring 10 μ in diameter.

Trematodes are flatworms, and they have a very complicated life cycle that involves one or two intermediate hosts. Fish may serve as the final host, and adult trematodes are commonly found in the digestive tract. However, it is as the intermediate host to the larvae (the migrating cercaria or metacercaria) that fish may transfer the parasite to man. Man's habit of eating raw or insufficiently cooked fish provides adequate opportunity for infection. Promiscuous or deliberate defecation into ponds, lakes and streams furnishes a constant supply of parasite eggs that, together with suitable intermediate hosts, continue the life cycle as shown in Figure 7.2.

Most trematodes of public health significance are found in tropical waters. *Clonorchis sinensis*, *Opisthorchis* sp., *Matagonimus*

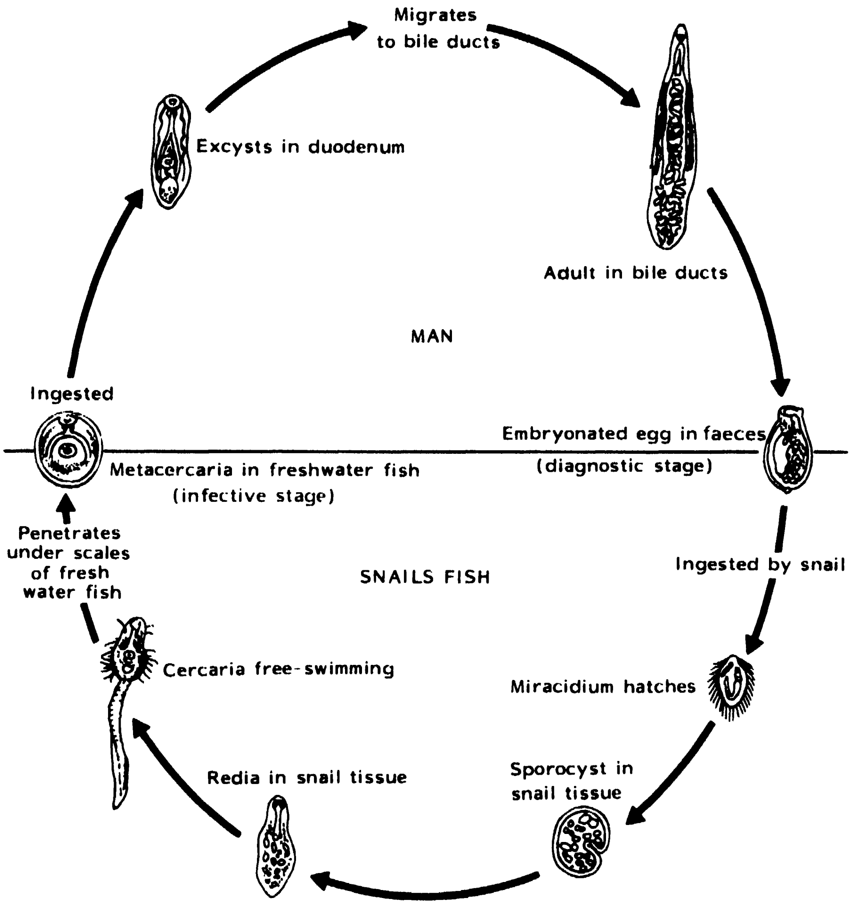


Figure 7.2. Life cycle of *Clonorchis sinensis* (Healy and Juranek, 1979)

yokagawai and *Heterophyes heterophyes* are all found in the Far East, but *H. heterophyes* is also found in the Nile delta and India. They all have a very similar life cycle as shown in Figure 7.2. *C. sinensis* and *Opisthorchis* sp. are very common liver flukes. They are formed in the bile duct of humans and cause inflammatory and fibrotic changes in bile duct, gall-bladder and liver. *H. heterophyes* and *M. yokagawai* are different in that they live in the intestines of humans and produce inflammation, symptoms of diarrhoea and abdominal pain.

Another trematode belonging to the genus *Paragonimus* is the very common oriental lung fluke. Humans are infected by eating raw or inadequately cooked crabs or crayfish. The adult parasite lives in cysts in the lungs, but it also has a tendency to migrate to other sites such as liver, spleen and brain. A chronic pulmonary disease ensues when the worms develop in the lungs.

Among the cestodes (tapeworms), only the *Diphyllobothrium*, the broad fish tapeworm, has a worldwide but dotted distribution. The biology of this group involves two types of intermediate host: copepods and fish.

If raw fish muscle or roe containing the living plerocercoids, which are non-encysted, glistening white worms, is injected into the intestines of the definitive mammalian host, the adult tapeworms that generally cause debility and anaemia develop.

Finally a number of nematodes (roundworms) may reach man when infected fish, molluscs or crustaceans are consumed. The adult worms live in the intestines, kidneys or other organs of fish-eating mammals or birds. The eggs of the worms must reach water to mature. Copepods, worms or molluscs are the first intermediate host, and fish, reptiles, birds and mammals serve as second and third hosts. In fish, the larval nematode is encysted in the abdominal cavity or in the lateral body muscle.

Some of the most common parasites in fish are nematodes of the genus Anisakidae, which may be 1-6 cm-long worms. Among these is the common *Phocanema decipiens*, the so-called cod worm, which also occurs in many other fish species. The occurrence of this large parasite represents a considerable marketing problem in many North Atlantic fisheries, since it renders the fish unsightly. Furthermore there is some indication that this parasite is a potential pathogen (Cheng, 1973) causing granulomas of the intestinal tract. Such symptoms have also been seen after infection with the common herring-worm (*Anisakis marinae*) (see Fig. 7.3).

Man is infected by eating raw or improperly processed fish. Slightly salted raw herring has been the most commonly reported source of infection.

Another nematode, *Angiostrongylus cantonensis*, is a common parasite in the lungs of rats in Southeast Asia. The parasite has been shown to cause meningitis in man. Its intermediate hosts are snails, freshwater prawns or land crabs.

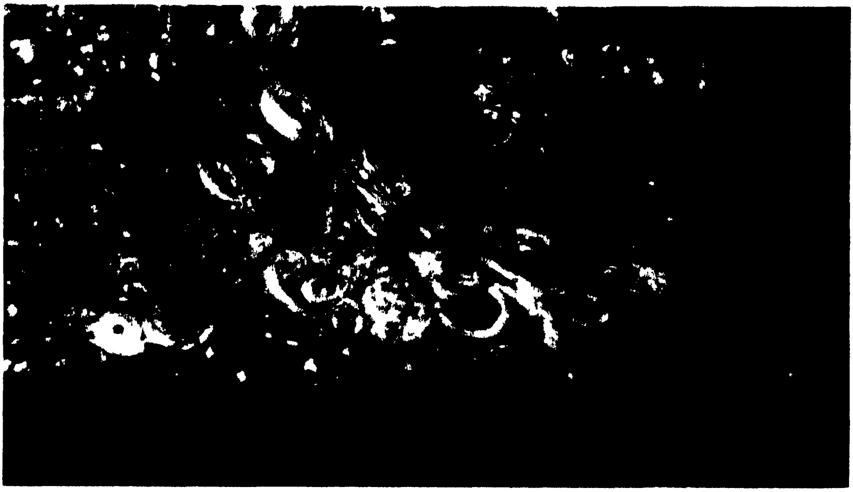


Figure 7.3. *Anisakis* larvae on the surface of herring ovaries

Biotoxins

More than 500 marine fish species are known to contain biotoxins, but only a few of these species are major sources of human food. However, these toxic fish pose serious problems in certain tropical and subtropical areas.

From both a public health and an economic point of view, ciguatera is the most serious of the marine fish toxins. More than 400, mostly tropical, fish species, including snapper (Lutjanidae) and grouper (Serranidae), have been identified as causing intoxication in man. It is possible that toxicity is associated with a component in the fish diet, but the precise cause is not known (fish may suddenly become toxic and remain so for years). The toxin is heat-stable and symptoms of intoxication include mild paralysis, gastrointestinal disturbances and, in extreme cases, death.

Tetraodon (puffer fish) poisoning is associated with consumption of fish commonly known as puffer, blow, globe and porcupine fish. These can be recognized by their characteristic shape, large teeth and distinctive odour of freshly dressed tissue. Most of them belong to the order Tetraodontiformes (80 species are toxic), and they are found worldwide in tropical zones. The heat-stable toxin is concentrated in the gonads and the liver. In Japan, where the fish are considered a delicacy, licensed chefs prepare *fugu* dishes from the flesh. These chefs are required to have a thorough knowledge of toxic species and seasonal variation in toxicity, and must be skilled in proper evisceration. Nevertheless, several hundred cases of intoxication are recorded in Japan each year, of which about half are fatal. Symptoms of poisoning, which appear within one hour of consumption, are numbness of the lips, tongue and fingers, often accompanied by nausea and vomiting. In the later phases of extreme cases, paralysis of the extremities, ataxia and loss of consciousness occur. Finally, death results from respiratory paralysis.

The oldest known shellfish-associated poison is the paralytic shellfish poison (PSP). It has been known for several hundred years in Europe and North America (Krogh, 1979), but recently an increasing number of outbreaks have been recorded. PSP is caused by molluscs which have ingested toxic dinoflagellates and which are subsequently eaten by man. The most common organisms associated with PSP are *Gonyaulax catanella* and *G. tamarensis*. Dinoflagellates are widely distributed, from polar oceans to the tropics. “Blooms” of toxic dinoflagellates occur during certain conditions, such as weather disturbances, heavy “wash-off” from land to coastal waters, high ambient temperatures and anoxic conditions at the bottom of shallow bays. The result is frequently referred to as “red tide”. Often there is also mass mortality of fish, which may occur from the toxic algae or the anoxic conditions.

The heat-stable toxin may accumulate in molluscs over a few days. These gradually destroy or excrete the toxin, but it may take several weeks or even months. The toxin, which is a saxitoxin, causes a tingling sensation in the mouth and lips, but larger doses may cause collapse, paralysis and death.

Apart from the poisons described above, it should be emphasized that food poisoning from eating certain kinds of fish is a danger which is always present.

Toxicity of a sporadic nature has been reported with many groups of fish and marine organisms. These include the cyclostomas (lampreys), elasmobranchs (sharks and rays), chimaeras (rat fish), clupeoids (e.g. herrings, anchovies, tarpons, etc.), mullet and marine turtles. The cause of this intoxication is not known. Also intoxication from eating organs of certain species (*Somniosus microcephalus*) has caused many outbreaks of disease, but the poison has not been identified. It is well known, however, that the liver from many

Arctic animals (polar bears, bearded seal, sea-lions) is toxic because of a very high content of vitamin A.

A special type of intoxication is the scombrotoxin poisoning caused by ingestion of fish from the Scombridae family (reviewed by Arnold and Brown, 1978). Typical fish species involved are tuna (*Thunnus thynnus*), mackerel (*Scomber japonicus* and *Scomber scombrus*), sardines (*Sardinops sagax*) and bonito (*Sarda chiliensis*). These fish may at times become poisonous as a result of improper preservation or faulty handling. The heat-stable toxin accumulates in the flesh during storage as a result of microbial action, and it is not destroyed during subsequent canning or smoking operations. Poisonous scombroid fish often have a sharp pepper-like taste. After consumption, symptoms of a severe allergy may develop within a few minutes. Typical symptoms are headache, dizziness and a "hot" flushing of the face and neck. Accounts of outbreaks have appeared regularly over the past 40 years from Japan, the Pacific islands and the United States, but over the last few years increasing numbers of incidents have been recorded in Europe and elsewhere.

The precise cause of scombroid poisoning is not known. However, the condition is always associated with high levels of histamine in the flesh.

Scombroid fish have characteristically high concentrations of the amino-acid histidine, which is transformed by a bacterial decarboxylase enzyme into histamine. This enzyme is found in bacteria belonging to the Enterobacteriaceae, particularly *Proteus morganii*, *Klebsiella* and *Hafnia* spp. These bacteria generally have a minimum growth temperature of around +8°C and grow very rapidly at temperatures greater than 15°C.

During storage of fish in ice, only small amounts of histamine are formed (∾3-4 mg/100 g fish); however, if the fish are stored at high ambient temperatures (15-20°C), large amounts of histamine are rapidly formed, and the fish may become toxic even though still acceptable to the consumer (Gilbert *et al.*, 1980).

Experience has shown that in outbreaks of scombroid poisoning the histamine content in the fish is normally above 20 mg/100 g fish, and this level has been suggested as an international standard.

However, histamine does not appear to be the sole causative agent in scombroid poisoning since histamine by itself is not toxic when taken orally. In a report by Bjeldanes *et al.* (1978) it is concluded that it is likely that cadaverine, along with histamine, is of importance in the aetiology of scombroid poisoning.

Chemicals

Chemicals may be found in tissues of fish and other predatory species as a result of biomagnification, which is the concentration of the chemicals in the higher trophic levels of the food chain. Or they may be there as a result of bioaccumulation, where increasing concentrations of chemicals in the body tissues accumulate over the life span of the individual. In this case, a big (i.e. older) fish will have a higher content of the chemical concerned than a small (younger) fish of the same species.

The first encounter with the potential danger of chemical contamination with food was in Minamata Bay in Japan in 1953. A mysterious neurological illness, which reached epidemic proportions (44 deaths and 111 cases of severe illness with disturbance of hearing and vision, and brain damage), turned out to be poisoning with methyl mercury. The mercury had drained into the bay from industrial waste and sewage systems, and fish caught in the bay were responsible for the disease. The Minamata incident was later (1962) repeated in Niigata, also in Japan.

As a natural element, mercury is distributed throughout the earth. However, the natural distribution is significantly altered by man since mercury is used in a variety of industrial and agricultural processes and is released via combustion of fossil fuel. The risk of environmental pollution in local areas is, therefore, high.

Numerous reports have concluded that the ocean levels of mercury have not changed significantly (Bradley and Hugunin, 1980), and the natural levels in seawater are the order of 0.01-0.02 ppb. In fish from the open sea, a level of 0.05-0.08 mg/kg has been found but ten-fold increases have been seen in fish from Scandinavian coastal waters (Engberg, 1976).

An FAO/WHO expert group has established a "provisional tolerable weekly intake" of 0.2 mg methyl mercury per person. Following this, a number of countries have imposed tolerances on foods. Thus the United States and Canada permit a maximum of 0.5 mg Hg/kg fish, but other countries may allow up to 1 mg Hg/kg.

So far there has been no registered case of food poisoning from any other heavy metal or hazardous substances derived from industrial sources. However, the public health aspects of environmental pollution are considered by relevant agencies in order to identify substances present in fish and shellfish that are a potential risk to man. This usually requires local investigations and monitoring of the environment and fish. Compounds of public concern are cadmium, cobalt, lead, pesticide residues (DDT) and polychlorinated biphenyls (PCB). It is stated by Coffin and McKinley (1980) that pesticides do not appear to cause any alarm. Because of restrictions on their use, pesticide levels in fish and the environment are decreasing (Fig. 7.4).

Fish diseases

There are no known bacterial or viral fish diseases which are transmissible to man. In fact, it is very rare to encounter diseases in fish in the

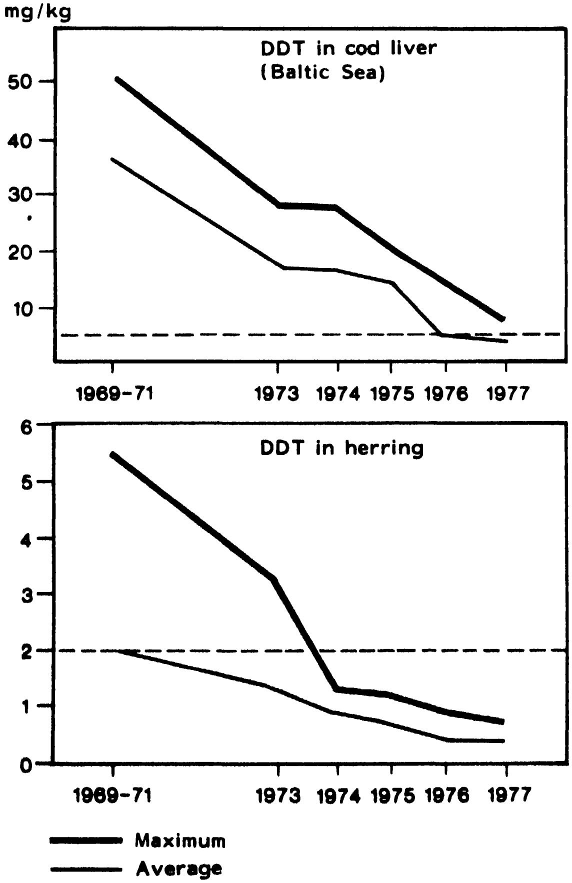


Figure 7.4. DDT in herring and cod liver from the Baltic (Danish National Food Institute)

Figure 7.5. Ulcus in cod (*Gadus morhua*). (The ulcer syndrome)

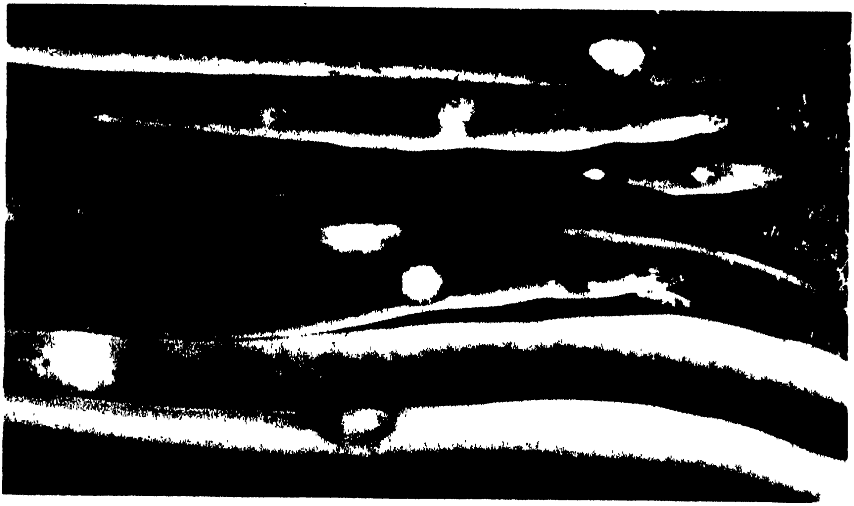


Figure 7.6. Ulcus in eel (*Anguilla anguilla*)

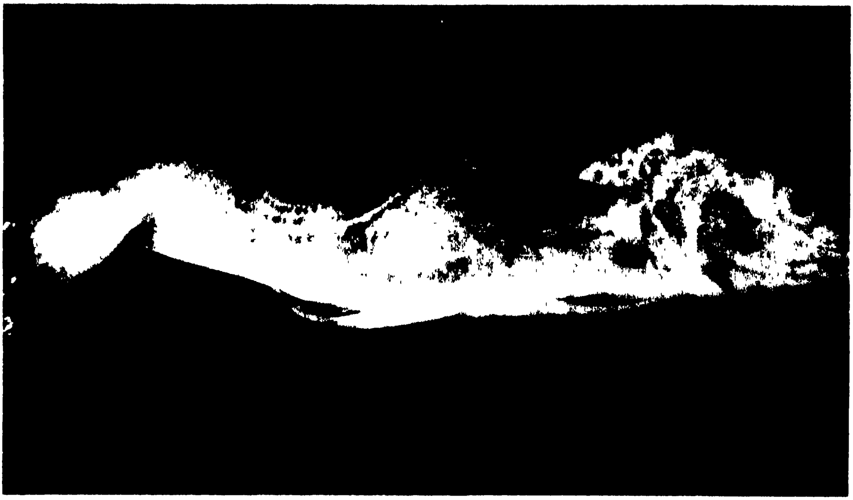


Figure 7.7. Skeletal abnormality in cod (*Gadus morhua*)

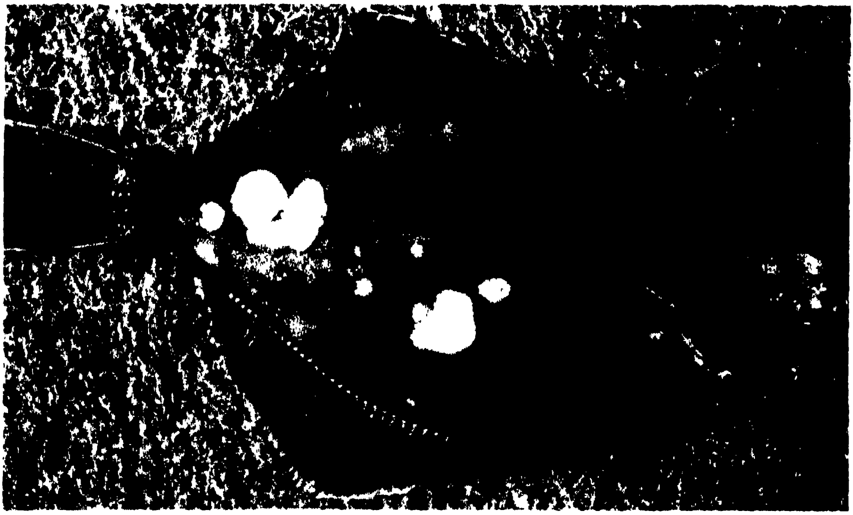


Figure 7.8. Papilloma in flatfish (*Pleuronectes platessa*)

open sea, although in coastal areas a large part of the fish population may be affected with various types of chronic diseases. Most frequently, various forms of ulcers may be seen (Fig. 7.5). It has been shown (Larsen *et al.*, 1978) that this disease in marine fish is probably caused by a virus followed by infection with *V. anguillarum*. In freshwater fish, infection by *Aeromonas hydrophila* may cause ulceration (Fig. 7.6). Other defects seen in fish are erosion of the fins, deformations of the skeleton (Fig. 7.7) and various forms of papillomatous and tumorous growth (Fig. 7.8).

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